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ORIGINAL ARTICLES

THE ACTION OF ETHYLENEDIAMINE*

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DESPITE the fact that the significance of the amino group in conferring pharmacologic activity has long been axiomatic, our knowledge of the action of the simple amines still remains very incomplete. One of these, ethylenediamine, the subject of this paper, is of interest both as a product of putrefaction, (a homologue of putrescine and cadaverine) and a constituent of certain antiseptics.

Ethylenediamine, $NH_2.\ CH_2.\ CH_2.\ NH_2$, has been known since its synthesis from alcoholic ammonia and ethylene bromide by Cloez, ('53).[1]

Brieger ('83)[2] isolated the base from putrefying fish, and Kulneff ('91)[3] from the gastric contents in a case of pyloric cancer. It has also been obtained by Carbone ('91)[4] from a growth of proteus vulgaris.

The first therapeutic appearance of ethylenediamine was as a constituent of "argentamin" (Schering and Schaeffer, 1894).[5] This antiseptic is a mixture of ten per cent each of silver phosphate and ethylenediamine.

Other antiseptics containing ethylenediamine are "kresamin," and "sublamin," in which it is combined with cresol, and with mercuric sulphate, respectively. Superior tissue penetrating powers are claimed for these preparations. (Schaeffer).[6]

PREVIOUS PHARMACOLOGIC STUDIES

Brieger[7] with "large doses" of the purified hydrochlorid produced in frogs a lethargic condition in which a ready response to stimuli persisted. Mice and

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guinea pigs were found less resistant than frogs to the action of ethylenediamine and reacted by a marked stimulation of nasal, ocular, and oral secretions. Dilation of the pupils and exophthalmia were also observed.

Rabbits gave similar results. In a half kilo rabbit Brieger "injected" 0.2 grams of the amine hydrochloride and observed the following symptoms: Hypersecretion from eyes, nose and mouth; pupillary dilation with poor response to light; pulse rate at first accelerated but later diminished; dyspnea; head drawn back in a spasm; marked twitching of the nose. After some hours the respiration returned to normal and muscular depression ensued. The animal made no voluntary movements but had not lost its reflexes. Autopsy findings were negative.

Schaeffer,[8] working with the base, describes ethylenediamine as having too slight a toxicity to interfere with its therapeutic application. He states that rabbits tolerated 0.4 gram of the base "without marked influence upon the health" provided that the concentration of the injected solution was not strong enough to cause corrosion or infiltration. He did not observe the effects upon the secretions and central nervous system noted by Brieger. White mice tolerated 30 milligrams of the diamine, larger doses causing death. Schaeffer apparently employed subcutaneous injections in all of this work.

Desgrez and Dorléans[9] report that ethylenediamine in common with hydrazine and monomethylamine lowers the blood pressure in rabbits and dogs. They claim, however, that large doses (4 mg.) raise the blood pressure in dogs.

In view of the paucity of previous work and the discrepancies between the work of Brieger and Schaeffer it was decided to reinvestigate the action of ethylenediamine. Kahlbaum's hydrochlorid was employed, its purity first being checked by melting point determinations of the picrate. Observations were made upon the toxicity for mice, the central nervous action in frogs, and the effects of single and repeated doses in rabbits. In addition studies were made of the effects upon smooth muscle, as well as on the circulation in anesthetized mammals.

TOXICITY

In rabbits single subcutaneous doses of 0.4 gram per kilo produced no alarming symptoms. Intravenously, however, this dose was fatal to two adult animals; the third recovered after treatment for respiratory failure, while the fourth tolerated a very slow injection.

In white mice, of three which received subcutaneously 12½ milligrams per 20 grams body weight, all survived, while there was but one survivor of three receiving 15 milligrams per 20 grams body weight (Table I). The latter figure equivalent to 0.75 gram per kilo expresses approximately the minimal lethal subcutaneous dose for mice. On the basis of its diamine content the hydrochlorid is therefore four times as toxic by subcutaneous injection as the base.[6] The symptoms noted in mice were depression, cyanosis, and respiratory failure.

TABLE I

MOUSE	WT. (GRAMS)	DOSE PER 20 GRAM WT. (MG.)	FATE
1	19.5	12.5	Recovered
2	19.5	12.5	"
3	16.5	12.5	"
4	20.5	15.0	Dead (60 hours)
5	21.0	15.0	" (100 hours)
6	23.0	15.0	Recovered

CENTRAL NERVOUS ACTION IN FROGS

The lethargic condition described by Brieger was found easy to reproduce in frogs (*R. pipiens*) with about 2 mgms. per gram body weight of the hydrochlorid. Contrary, however, to the impression gained from Brieger's report, the spinal cord becomes depressed as well as the higher centers.

The following typical protocol (Table II) shows the prolongation of the reflex-time. The stimulus employed was 0.5 per cent hydrochloric acid into which the toes were dipped for a distance of 5 mm. The brains of two frogs were pithed and the animals suspended by the lower jaws. One was observed as a control, the other receiving 70 mg. of the diamine in the anterior lymph sac.

TABLE II

PITHED FROGS

TIME	CONTROL FROG NO. 1, 32 GRAMS	ETHYLENEDIAMINE—HCl FROG NO. 2, 34 GRAMS	
1:33 P.M.	———	70 mg. injected	
2:15 P.M.	1.5 seconds	1.5 seconds	
2:45 P.M.	1.5 "	6.0 "	
3:15 P.M.	2.0 "	30.0 "	No response Heart still beating

In experiments upon intact frogs prolongation of the reflex time was always noted before depression of the respiratory movements. Its action therefore conforms in type to that of the methane narcotics (paralysis of cerebrum → cord → medulla).

ACTION UPON RABBITS

The effects of single and repeated injections upon pulse, respiration, temperature, secretions, and general behavior were investigated. Where the injections were repeated daily for ten days, observations were also made upon the amount and character of the urine and the changes in body weight.

All of the rabbits were healthy and were kept upon a quantitative diet of oats, carrots and water, for at least one week previous to the observations.

Large Doses.—Seven rabbits were given ethylenediamine hydrochloride in single doses of 0.4 gm. per kilo. (Nos. 1 to 5 intravenously, and Nos. 6 and 7 subcutaneously.)

Given by vein this dose proved immediately fatal to two animals. A third was saved only by artificial respiration. The other two, and the two which received subcutaneous injections, exhibited certain constant effects. A very brief general depression was observed together with a short lasting increase (approxi-

mate doubling) in the number of the respirations. The body temperature, with one exception, was considerably reduced, recovery requiring a number of hours. Neither narcosis nor depression of reflexes was observed.

Secretions.—In spite of careful search the stimulation of lacrimal, nasal or salivary secretions, reported by Brieger, were never observed. Furthermore, a 270 gram guinea pig received subcutaneously 0.1 gram of the hydrocloride* but the only resulting indication of hypersection was a marked diarrhea.

Heart.—The protocols show that ethylenediamine does not affect the pulse rate in rabbits.

Blood.—Rabbit's blood taken shortly after the administration of 0.4 gram per kilo of ethylenediamine exhibits no abnormalities in hemoglobin content or in the appearance of the red blood cells. Methemoglobin is not produced.

Fig. 1.—Action of 10 mg. ethylenediamine hydrochloride upon isolated cat's intestinal muscle suspended in 50 c.c. oxygenated Locke's solution.

Ten mg. diamine added at mark. Cessation of activity and relaxation of tone followed within about five minutes by spontaneous recovery.

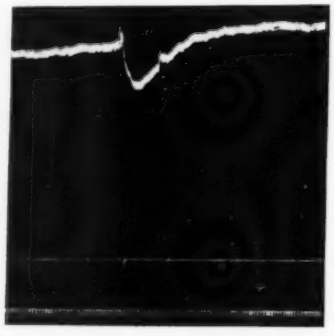

Fig. 2.—Intravenous injection of 10 mg. ethylenediamine hydrochloride 2 kilo rabbit.
1. Blood pressure. 2. Base line zero pressure. (Injection at the mark.)
3. Time in 5 second intervals.

Local Reaction.—Subcutaneous injections of ethylenediamine hydrochloride in the doses named produced neither abcesses nor other inflammatory reaction at the site of injection.

Intestinal Action.—Diarrhea was noted in every case within a few hours after the administration of ethylenediamine.

In Rabbits 5 and 6 the urine was examined for ethylenediamine by the periodide test of Pohl,[10] the results being negative both on the day of injection and the following day in both cases.

Repeated Small Doses.—Two rabbits received subcutaneous injections of 0.1 gm. per kilo daily for a period of ten days. The only significant effect was

*This animal showed marked muscular depression and a 20 per cent increase in the respiratory rate.

the reduction of the body temperature which, as was usual after the larger doses, amounted to 1° C. or more. Both of the animals acquired a tolerance to the temperature reducing effect, the fall becoming less each day until after the fourth day when it was missed altogether.

Other observations upon these animals included the following: A loss of 50 gm. weight in ten days by one animal, the other's weight remaining unchanged. Variations in volume, specific gravity, and cell content of the urine in both cases were within normal limits throughout. The drug never produced albuminuria or glycosuria. Only one of these two animals exhibited diarrhea.

ACTION UPON SMOOTH MUSCLE

A number of strips of excised cat's intestine were tested by the usual method for studying the action of drugs upon smooth muscle. Complete cessation of activity and relaxation of tone was readily produced, recovery occurring spontaneously after a few minutes. Ten and 5 mg., respectively, of the hydrochloride in 50 c.c. of oxygenated Locke's solution sufficed to produce these inhibitory effects. (See Fig. 1.) One mg. was ineffective.

ACTION UPON THE CIRCULATION

Administered intravenously ethylenediamine hydrochloride produces a transitory fall in the blood pressure of anesthetized animals. This action was observed in rabbits, cats, and dogs, confirming the results of Desgrez and Dorléans,[9] and was quite marked even with doses as low as 1 mg. The pressor effect described by these authors in dogs after larger doses was, however, not obtained. The subcutaneous injection of 100 mg. in a 4 kilo dog failed to alter the blood pressure at all. Fig. 2 illustrates the effect of a 10 mg. injection intravenously upon a 2 kilo rabbit under urethane.

Regions of Cardiovascular System Affected.—In many experiments the pulse rate was determined before and after injection. As in the case of the intact animals, no significant change was observed. The blood pressure fall therefore appears to be independent of the heart's action.

It was next attempted to ascertain whether or not the depressor effect is limited to definite areas such as the extremities or the viscera. For this purpose rabbits were anesthetized and the blood supply to various regions excluded by arterial ligation. By clamping the abdominal aorta below the renal arteries, the subclavian arteries, and the unused carotid artery, the circulation in the extremities and part of the head was eliminated. After this procedure (Fig. 3, *y*) 10 mg. of ethylenediamine caused a slightly greater fall in the blood pressure than was produced previous to the ligations (Fig. 3, *x*).

In another rabbit when the mesenteric arteries were ligated the diamine action remained unaltered (Fig. 4). In the same animal later the celiac, mesenteric and subclavian arteries and abdominal aorta were clamped and the drug administered as before. The depressor effect, although somewhat more gradual in onset and prolonged, was still obtained (Fig. 5). The presence of the splanchnic circulation is therefore by no means essential to the diamine effect, in fact the combined response of the vessels of the thorax, the superficial vessels of the

Fig. 3.

Fig. 3.—Intravenous injection of 10 mg. ethylenediamine hydrochloride in a 2300 gram rabbit before and after exclusion of the circulation of the extremities. 1. Blood pressure. 2. Base line. (Injection marks x and y. A. Aorta clamped. B. Subclavian arteries clamped. C. Unused carotid artery clamped.) 3. Time in 5 second intervals.

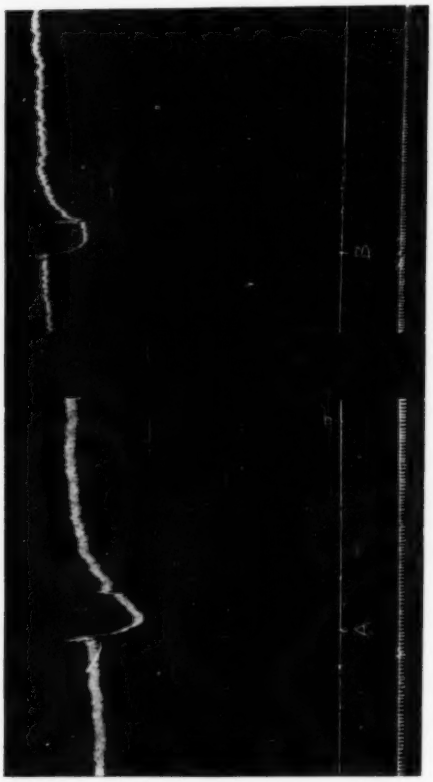

Fig. 4.

Fig. 4.—Two kilo rabbit. Ten mg. ethylenediamine hydrochloride (intravenously) before (A) and after (B) clamping the mesenteric arteries. 1. Blood pressure. 2. Base line. 3. Time (5 second intervals).

upper abdomen, and the vertebrals was nearly as extensive as that of the intact circulation. The depressor action of the drug is thus shown to be independent of any one of the chief regions of the vascular system.

Depression of Sciatic Pressor Reflex.—By an intravenous injection of ethylenediamine in a dog it was found possible to abolish partially for a time the

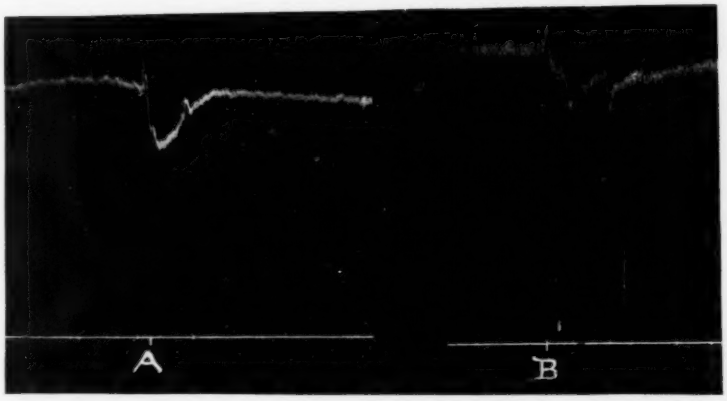

Fig. 5.—Same rabbit as in Fig. 4. Ten mg. ethylenediamine-HCl (intravenously), before (A), and after (B) exclusion of mesenterics, subclavians, and lower abdominal aorta.

1. Blood pressure. 2. Base line.

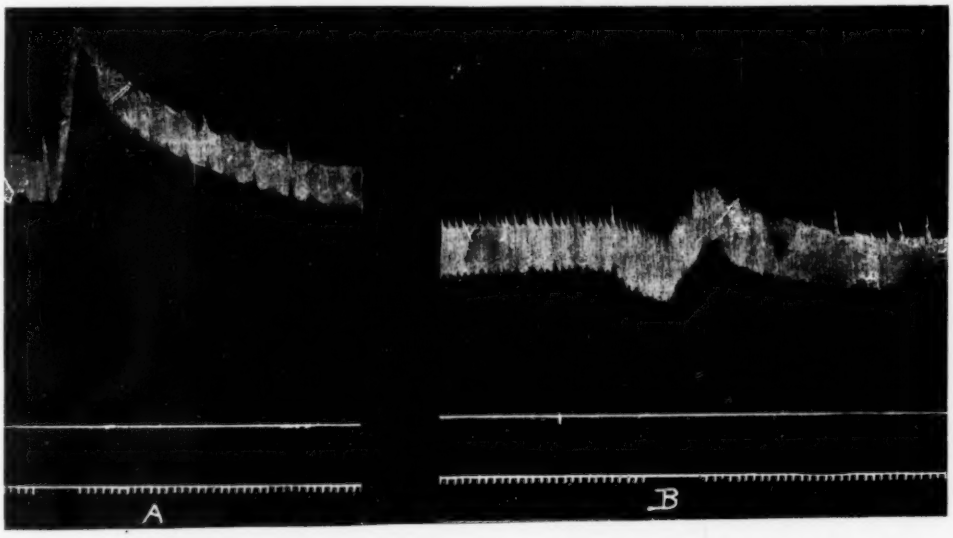

Fig. 6.—Effect of ethylenediamine (intravenously) upon the sciatic Pressor reflex in a 4 kilo dog. A. Effect of stimulation of the central end of cut sciatic. B. Same after injection of 20 mg. ethylenediamine at mark.

1. Blood pressure tracing. 2. Base line. 3. Time (5 second intervals).

sensitivity of the vascular system to reflex stimulation, as the following experiment shows.

The sciatic nerve of a 4 kilo dog under chloretone anesthesia was cut and the central end then stimulated by the induction current. After the resulting reflex blood pressure rise had been recorded (Fig. 6, *A*) 20 mg. of the diamine

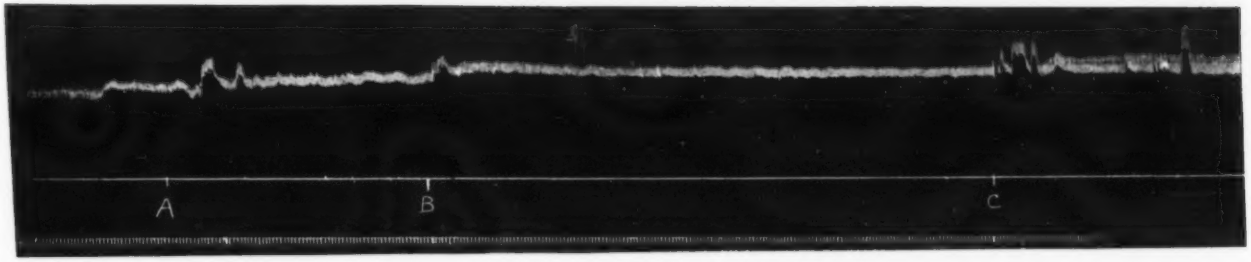

Fig. 7.

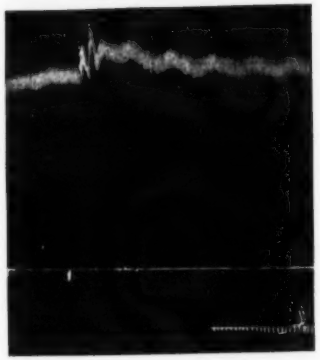

Fig. 8.

Fig. 7.—Effect of ethylenediamine hydrochloride (intravenously) on pithed cat. A. 45 mg. of ethylenediamine-HCl in 3 c.c. saline. B. 3 c.c. saline. C. 45 mg. of diamine in 3 c.c. saline.

1. Blood pressure. 2. Base line 3. Time (5 second intervals).

Fig. 8.—Effect of intravenous injection of 40 mg. of ethylenediamine hydrochloride in a nicotinized cat.

1. Blood pressure. 2. Base line. 3. Time (5 second intervals).

was injected and the nerve again stimulated with an equal current strength just as the blood pressure was beginning to fall. Only a slight response to this second stimulation was obtained (Fig. 6, *B*). The sensitivity returned after a few minutes and the entire experiment was then repeated with the same result.

Is the depressor action central or peripheral? This is always a difficult point to determine. The question was submitted to two methods of attack: we determined the action of ethylenediamine, (1) after the elimination of the vasomotor centers by pithing; and (2) upon the nicotinized animal.

(1) After elimination of the vasomotor centers by pithing, no fall in blood pressure can be obtained by intravenous injections of the diamine. Three rabbits injected under such conditions gave entirely negative results. Fig. 7 illustrates the effect of two injections of ethylenediamine (and a saline control) in a pithed

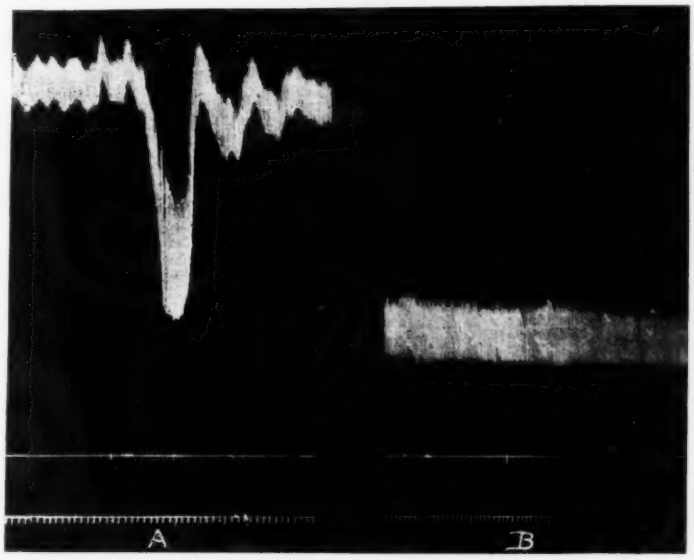

Fig. 9.—Effect of ethylenediamine (20 mg.) on the blood pressure of a dog, before (A) and after (B) nicotinization. Injection at marks.

1. Blood pressure curve. 2. Base line. 3. Time (5 second intervals).

cat, the only responses being slight increases in blood pressure. This type of experiment is unsatisfactory because of the marked circulatory depression produced by the pithing procedure itself. The results indicate, however, that the depressor action of the diamine is a central one.

(2) A number of rabbits were thoroughly nicotinized (until no sciatic pressor reflex could be obtained with maximal stimuli), after which ethylenediamine was impotent to affect the blood pressure. In Fig. 8 is illustrated the effect of an injection of 40 mg. of ethylenediamine in a nicotinized cat. One sees a blood pressure rise similar to that obtained in the pithed cat. A dog also gave a negative result; in Fig. 9 is seen the effect of 20 mg. of the diamine before (*A*), and after (*B*), nicotinization.

In view of the consistent failure to obtain any trace of depressor response to

ethylenediamine after decerebration or thorough nicotinization it is concluded that the central nervous system plays at least a large part in the blood pressure reducing effect of ethylenediamine.

CONCLUSIONS

1. Ethylenediamine hydrochloride is a comparatively innocuous substance, the minimal lethal subcutaneous dose for white mice being 15 mg. per 20 gm. body weight.

2. Upon the central nervous system of frogs its action resembles that of the methane narcotics.

3. Rabbits exhibit after nearly fatal intravenous or subcutaneous injections, (0.4 gm. per kilo), a temporary respiratory stimulation and a lowering of body temperature. A slight general depression and transient diarrhea are also produced. Lacrimal, nasal, and salivary secretions are not stimulated. (Contrary to the results of Brieger.)

4. The only constant effect of smaller subcutaneous doses (0.1 mg. per kilo) is the hypothermia. Daily injections render the animals immune to this effect after four days. Continued for ten days such injections have no significant effects upon body weight, kidneys, heart or blood.

5. Ethylenediamine inhibits the activity and relaxes the tone of smooth muscle (isolated cat's intestine).

6. The blood pressure is lowered in dogs, cats and rabbits by intravenous injections of ethylenediamine, 1 mg. often producing this effect. This effect is not of cardiac origin neither is it limited to any particular region of the vascular system. During its exhibition the sciatic pressor reflex is partially abolished.

7. Ethylenediamine fails to lower the blood pressure after pithing or complete nicotinization. Its depressor action is probably of central nervous origin.

PROTOCOLS

Rabbit 1. 850 grams.
Single intravenous injection of ethylenediamine-HCl.
0.4 gram per kilo.

TIME	RESP. PER ¼ MIN.	RECTAL TEMP. °C.	PULSE PER ¼ MIN.
12-12-16			
9:34 A. M.	12	39.0	39
10:38	Injection		
10:41	28		39
11:00	15		
11:20	17	38.6	
2:00[1] P. M.	13	39.0	38
3:00	11	39.3	37
4:00[2]	14	39.5	39
12-13-16			
9:00[3] A. M.	14	39.3	39
12-14-16			
8:30[4] A. M.	14	39.5	39

1. Diarrhea—No other increased secretions. Pupils dilated, poor accommodation. Bright red urine; many r.b.c.
2. Bright red urine—abundance of r.b.c. and albumin.
3. Hematuria, albuminuria.
4. No hematuria, albuminuria.

Rabbit 2. 1990 grams.
Single intravenous injection of ethylenediamine-HCl.
0.4 gram per kilo.

TIME	RESP. PER ¼ MIN.	RECTAL TEMP. °C.
3-26-17		
12:30 P.M.	17	39.0
2:58	Injection	
3:00	Collapse. Resp. failure	Recovery by artificial resp.
3:10	33	
3:45	16	38.3
4:30	13	38.7
7:15	14	38.0
3-27-17		
9:15 A.M.*	16	39.3

*Diarrhea.

Rabbit 3. 1940 grams.
Single intravenous injection of ethylenediamine-HCl.
0.4 gram per kilo.

TIME	RESP. PER ¼ MIN.	RECTAL TEMP. °C.
3-26-17		
12:30 P.M.	10	39.0
3:20 } 3:25 }	Slow injection	
3:30[1]	20	
3:45	11	38.3
4:30	11	38.1
7:15	12	37.8
3-27-17		
9:15[2] A.M.	8	38.9

1. Slight depression, contracted pupils.
2. Diarrhea.

Rabbit 4. 1185 grams.
Single intravenous injection of ethylenediamine-HCl.
0.4 gram per kilo.
1-15-17 Died during injection.

Rabbit 5. 1105 grams.
Single intravenous injection of ethylenediamine-HCl.
0.4 gram per kilo.
1-15-17 Died during injection.

Rabbit 6. 1510 grams.
Single subcutaneous injection of ethylenediamine-HCl.
0.4 gram per kilo.

TIME	RESP. PER ¼ MIN.	RECTAL TEMP. °C.	PULSE PER ¼ MIN.
1-15-17			
3:55 P.M.	15	39.7	40
4:00	Injection		
4:45*	20	38.9	40
5:15	19	38.5	44
7:15	13	39.0	40
1-16-17			
9:35 A.M.	14	39.8	40

*Diarrhea.

Rabbit 7. 1485 grams.
Single subcutaneous injection of ethylenediamine-HCl. 0.4 gram per kilo.

TIME	RESP. PER ¼ MIN.	RECTAL TEMP. °C.	PULSE PER ¼ MIN.
1-15-17			
4:25 P.M.	10	39.4	42
4:30	Injection		
4:55*	20	39.6	44
5:25	18	39.6	42
7:20	12	39.4	42
1-16-17			
9:55 A.M.	11	39.5	42

*Severe diarrhea.

Rabbit 8.
Subcutaneous injections of ethylenediamine-HCl. 0.1 gram per kilo daily.

TIME	ETHYLENEDIAMINE GM.	RESP. PER ¼ MIN.	RECTAL TEMP. °C.
3-26-17x			
12:30 P.M.		12	39.5
2:45	0.173		
3:45		12	37.9
4:30*		12	38.1
7:15		12	39.6
3-27-17			
9:15 A.M.		14	39.5
2:45 P.M.		12	39.9
3:20	0.173		
4:20		11	38.6
6:45		12	39.3
3-28-17			
3:00		12	39.5
3:25	0.173		
4:30		12	38.8
3-29-17			
1:00		12	39.5
1:05	0.173		
3:00		12	39.3
3-30-17			
12:00		12	38.5
12:15	0.173		
12:30		12	38.6
12:45		10	38.4
1:00		12	38.3
2:00		11	38.7
3-31-17	0.173		
4-1-17	0.173		
4-2-17	0.173		
4-3-17	0.173		
4-4-17x			
11:30 A.M.		10	39.3
11:45	0.173		
12:45 P.M.		11	39.0

*Severe diarrhea.
xWeight first and last days, 1730 gms.

Rabbit 9.
Subcutaneous injections of ethylenediamine-HCl.
0.1 gram per kilo daily.

TIME	ETHYLENEDIAMINE GM.	RESP. PER ¼ MIN.	RECTAL TEMP. °C.
3-26-17*			
12:30 P.M.		12	39.5
2:50	0.178		
3:45		12	38.5
4:30		12	39.0
7:15		12	39.8
3-27-17			
2:45 P.M.		12	39.5
3:25	0.178		
4:20		16 irreg.	39.0
6:45		15	39.8
3-28-17			
3:00		12	39.5
3:30	0.178		
4:30		14	39.3
3-29-17			
1:00		14	39.1
1:10	0.178		
3:00		16 irreg.	39.3
3-30-17			
12:00		14	39.0
12:18	0.178		
12:30		20 irreg.	39.0
12:45		16 "	38.8
1:00		16 "	38.8
2:00		16 "	39.5
3-31-17	0.178		
4-1-17	0.178		
4-2-17	0.178		
4-3-17	0.178		
4-4-17†			
11:30 A.M.		12 irreg.	39.1
11:45	0.178		
12:45 P.M.		14 "	39.2

*Weight 1780 grams.
†Weight 1730 grams.

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CHEMICAL CHANGES IN THE BLOOD IN DISEASE*

II. Uric Acid

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MORE than seventy years ago Sir A. B. Garrod[1] put the subject of the uric acid content of the blood on a definite basis when he identified this substance in the blood of patients suffering from gout, and showed that whereas uric acid was normally present in blood only in traces, it was definitely increased, not only in gout, but also in certain cases of nephritis. He further showed that there is no increase in the blood uric acid in rheumatism, such as is found in gout, and used this as a point of differential diagnosis. No noteworthy advance in this subject was made until Folin and Denis[2] in 1913 introduced their simple colorimetric method for the estimation of this interesting substance. As originally carried out this method was not entirely accurate, but with the modifications since introduced, the method has been considerably simplified and the accuracy greatly improved.

Before considering the results which have been obtained with present methods, further reference should be made to the very interesting work of Garrod, since the general harmony of his conclusions with current views is surprising, when one considers the methods which were then available. It has been suggested that he drew upon his imagination for some of his results, but the correctness of his deductions and the quantitative data which he gives do not support this. This criticism probably originated from the fact that in his later clinical work he endeavored to gauge the amount of uric acid present in the blood by his famous thread test ("uric acid thread experiment").[3] This test was checked against tests where known amounts of uric acid had been employed. In his earlier work, however, the uric acid (or urates) obtained from a given amount of blood, 65 c.c. (1000 grains) was weighed. Such figures as the following recalculated as mg. per 100 c.c. were obtained: in gout, 5, 5, 2.5, 4.5, 3.0 and 17.5; in rheumatism, trace in four and negative in fifth case; in albuminuria, 0.5, 1.2 and 2.7 mg.; and in headache cases, 0.7, trace and 1.0 mg. Sheep and pigeon blood yielded negative results.

The method employed[1] was to evaporate the serum to dryness in thin layers in a water-bath. It was then powdered and treated with rectified spirits, boiled for about 10 to 15 minutes and again treated in the same way. After again washing with spirits, the dried serum was exhausted by means of boiling distilled water, the operation being repeated two or three times, and the watery solutions mixed. The concentrated watery solution was allowed to stand for some hours (forty-eight), when on examination, innumerable tufts of crystals were found deposited on the sides of the vessel, and the surface of the liquid. The crystals were collected, washed with alcohol, and weighed. "These crystals were proved to consist of urate of soda; for crystallized uric acid could be produced from them, and they left an alkaline ash, soluble in water and not consisting of potash."

It is of interest to note that in connection with his later determinations, Garrod writes:[1] "With regard to the weights which have been given, I may observe that in the earlier determinations they doubtless were below the real quantities, a circumstance which arose from the watery solutions of the serum not being sufficiently concentrated, and from sufficient time not being allowed for the deposition of the uric acid. In the experiments

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now made, I do not collect the crystals until forty-eight hours have elapsed. Such slight errors are, however, unavoidable in new investigations on any subject."

In the course of discussions in his several papers and book, Garrod makes many very interesting and pertinent remarks, a few of which will be quoted: "I may remark, that during evaporation of the serum of blood in albuminuria, a peculiar *odor* of urine was frequently detected; this was not observed in healthy serum, or in that taken from gout or rheumatic patients. Some of the coloring matters of the urine seemed, however, to be thrown down with the uric acid in all cases. * * * The results of these experiments on the condition of the blood and urine prove that uric acid is not a product of the action of the kidneys, as is frequently supposed, but that it is merely excreted from the system by these organs. * * * It appears also probable that as, in albuminuria, the 'urea-excreting function' being chiefly impaired, we find a vicarious discharge of this body in the dropsical effusions; so in gout, the 'uric acid-excreting-function' being defective, the chalk-like deposits are produced, by a similar vicarious discharge of urate of soda. * * * Gout would thus appear partly to depend on a loss of power (temporary or permanent) of this 'uric acid-excreting-function' of the kidneys; the premonitory symptoms, and those also which constitute the paroxysm, arising from an excess of this acid in the blood, and from the effort to expel the 'materies morbi' from the system. * * * In conclusion, as we have found that the blood in every patient suffering from genuine gout, contained an abnormal amount of uric acid, and that in acute rheumatism such was not the condition of this fluid; and again, that in all cases which could be traced up to gout (although the symptoms exhibited at the time might not be very characteristic), uric acid was present, whereas it was absent in those cases where no such phenomena could be found, I think we shall in future be justified in considering the condition of the blood as not only most important, but even a *pathognomonic* sign, and one more to be depended on than any of the other symptoms taken separately." In studying the therapeutic action of colchicum Garrod[4] noted no increase in the uric acid output or influence on urea or other solids of the urine, and observed that this drug did not act as a diuretic in all cases, a diminished output of urine sometimes being noted. He did not confine his attention entirely to uric acid for in a study of blood and effused fluids he writes:[5] "Lastly, these effused fluids may be employed, not only to ascertain the existence of uric acid, but likewise of other principles, as urea and sugar, which are contained and can be detected in them."

As noted above, little advance was made from the time of Garrod until Folin and Denis reported their first results[6] on the uric acid content of human blood. In a series of unselected cases they found between 1 and 3 mg. to 100 c.c., the average being close to 2 mg., while in gout their figures varied between 3.5 and 5.5 mg. Somewhat similar increases were observed in lead poisoning and leucemia. Practically no elevation of the uric acid was noted in a series of 11 nephritic bloods with only moderate nitrogen retention, but later they reported data[7] on cases of advanced nephritis in some of which very high values were obtained, up to 10 mg. These latter observations were confirmed by Myers and Fine,[8] who noted very high figures for uric acid in several cases of terminal interstitial nephritis. In one case the uric acid reached the enormous figure of 27 mg. shortly before death (see Table I), while in several cases figures as high as 15 mg. were observed, values much higher than any noted in gout.

The figures which are now regarded as normal for the blood uric acid differ very little from those originally reported by Folin and Denis. Although healthy adults most often yield values between 2 and 3 mg. per 100 c.c. of blood, figures as low as 1 mg. and as high as 3.5 mg. may be encountered in strictly normal individuals, the differences probably depending in part upon dietary

factors. High blood uric acids must obviously depend upon either an increased formation or a decreased elimination. In leucemia the first factor accounts for the increase, but high uric acids in most other conditions find a probable explanation on the latter basis. Among these may be mentioned nephritis, acute and chronic (but not parenchymatous), arterial hypertension, lead poisoning, bichloride poisoning, malignancy, acute infections, especially pneumonia, and apparently some cases of nongouty arthritis. Miscellaneous cases illustrating the uric acid findings in most of these conditions are given in Table III of the preceding paper, and in Table II here. Sedgwick and Kingsbury[9] have made the interesting observation that the blood uric acid is high during the first three or four days of life, in harmony with the high uric acid excretion during that period.

NEPHRITIS

It is perfectly logical to expect that high values for the blood uric acid would be found in the last stages of chronic interstitial nephritis, with the consequent accumulation of all the waste products of nitrogenous metabolism. Comparative figures on the blood and urine in one of our first cases[8] are given in Table I. The blood uric acid findings in this case are higher than in any case

TABLE I

COMPARATIVE BLOOD AND URINE FINDINGS IN CHRONIC INTERSTITIAL NEPHRITIS

I. D., female, aged 17

DATE 1914-15	BLOOD ANALYSES MG. TO 100 C.C.				DATE 1914-15	URINE ANALYSES DAILY AVERAGES IN GMS.		
	Nonprotein N	Urea N	Uric Acid	Creatinine		Total N	Uric Acid	Creatinine
Dec. 10	181	139	6.8	10.0	Dec. 18-19	5.57	0.14	0.37
Dec. 21	199	134	12.5	14.5	Dec. 20-23	3.65	0.07	0.29
Dec. 26	244	151	15.4	17.7	Dec. 23-26	3.64	0.16	0.17
Dec. 30	267	170	21.0	16.1	Dec. 26-30	2.91	0.13	0.13
Jan. 4	297	208	27.0	20.0	Dec. 30 to Jan. 3	1.75	0.09	0.15

we have since studied or any elsewhere reported. Indeed, we might have had some doubts about the last findings recorded, were it not that some of the observations were checked in another laboratory. That the retention of uric acid in nephritis results in a fairly even distribution of this substance in the various body tissues has been shown by Fine[10] in tissues obtained at autopsy. The distribution, however, is not quite as uniform as in the case of the urea or even the creatinine, a fact which might be expected from their physical properties.

In 1916 Myers, Fine and Lough[11] called attention to the fact that very high figures for uric acid may be noted, not only in cases of advanced interstitial nephritis, but also in the very early stage of the disease, before a retention of either the urea or creatinine had taken place. It was suggested that, when symptoms of gout were absent, a high blood uric acid might be a valuable early diagnostic sign of nephritis, possibly earlier evidence of renal impairment of an interstitial type than the classic tests of proteinuria and cylinduria.

Subsequently, Baumann, Hansmann, Davis and Stevens[12] took up a study of this question and concluded: "It follows from the above that the uric acid concentration of the blood is a delicate, if not the most delicate, index of renal function at our disposal." More recently Upham and Higley[13] have taken up the renal concentration power for uric acid. It was pointed out by Myers and Fine[14] that under normal conditions the kidney concentrates the uric acid only about twenty times, whereas in the case of urea and creatinine the figures were about 80 and 100, respectively. Upham and Higley have found that in cases free from clinical symptoms of nephritis the concentration power for uric acid was 20 or over, while in cases showing clinical symptoms diagnostic of nephritis, it was 14 or below. In another group of cases showing symptoms suggestive but not diagnostic of nephritis the concentration figure was 18.4 or below.

TABLE II

ILLUSTRATIVE URIC ACID FINDINGS IN NEPHRITIS, LEUCEMIA AND GOUT

CASE	AGE	SEX	DATE	BLOOD ANALYSES MG. TO 100 C.C.			CLINICAL DIAGNOSIS, REMARKS
				Uric Acid	Urea N	Creatinine	
1. H. B.	55	♂	3/28/16	6.1	16	2.8	Chronic diffuse nephritis; hypertension.
2. B. D.	25	♀	3/15/16	9.6	19	2.4	Chronic diffuse nephritis; hypertension and edema.
3. D. S.	56	♂	6/ 7/15	7.1	16	2.0	Chronic interstitial nephritis.
			7/21/15	6.6	24	3.3	
			9/28/15	6.3	18	2.1	
4. J. J.	65	♂	8/11/15	9.5	25	2.5	Chronic interstitial nephritis; died four years later.
			9/25/15	8.0	37	2.7	
			1/14/16	5.0	37	3.9	
5. M. C.	11	♂	6/27/16	8.0	36	2.7	Chronic diffuse nephritis; improvement.
			7/21/16	6.3	53	2.9	
6. W. C.	49	♂	1/15/16	9.5	44	3.5	Acute nephritis; acidosis; recovery.
			1/28/16	2.5	19	1.9	
7. F. F.	8	♂	12/18/17	11.4	106	6.1	Acute nephritis; suppression of urine; recovery.
			12/21/17	11.2	93	5.9	
			12/27/17	5.0	21	4.2	
8. G. McK.	21	♀	12/ 4/19	9.3	48	13.4	Terminal chronic interstitial nephritis.
9. C. P.	37	♂	3/19/20	7.7	111	21.7	Terminal chronic interstitial nephritis.
10. J. P.	62	♂	4/ 4/16	3.4	28	3.0	Chronic parenchymatous nephritis; died.
11. S. R.	13	♂	8/15/15	10.0	17	2.4	Lymphatic leucemia; died.
12. H. L.	51	♂	3/25/16	7.8	12	2.9	Typical gout for 22 years; tophi on ears; severe pains in joints, particularly right big toe; diet purine-free until after second test.
			4/14/16	6.4	11	2.2	
			5/ 5/16	8.2	15	2.3	
			5/10/16	8.1	15	1.5	

An idea of the uric acid findings in illustrative cases of nephritis may be obtained from Table II. The first two cases show that it is possible to have

very high figures for uric acid without any very definite retention of urea. At a later stage in the disease the urea retention becomes definite as is seen in Cases 3, 4, and 5. Acute nephritis may influence not only the uric acid but also the urea and to some extent the creatinine. This is well illustrated by Cases 6 and 7, the first of which showed pronounced acidosis. Note the marked fall in the uric acid following improvement in this case. The nitrogen retention in Case 7 is the most pronounced we have observed with ultimate recovery. The figures on Cases 8 and 9 were obtained in the last stages of the disease, and show a retention of the urea and creatinine as well as of the uric acid. It is of interest regarding the uric acid that early in the disease the values observed may be somewhat higher (7 to 8 mg.) than at a later stage (5 to 6 mg.), due possibly to dietary restrictions, although during the last days of life the amount may be very markedly increased. In parenchymatous nephritis there is very little retention of uric acid.

GOUT AND ARTHRITIS*

Owing to the fact that the tophi found in gout have long been recognized to contain deposits of sodium urate, it is quite natural that the uric acid content of the blood in this condition should possess a special interest. Following the investigations of Folin and Denis a number of different workers took up a study of this question, among whom may be mentioned McLester,[15] Pratt,[16] Fine and Chace,[17] and Daniels and McCrudden.[18] Subsequently Folin and Denis[19] and Fine[20] further discussed this question, while recently McClure in association with several workers[21, 22, 23, 24] has given considerable attention to this subject. His last paper gives an excellent presentation of his own work.[24] Although the method originally described by Folin and Denis[2] yielded fairly satisfactory results when carefully executed, many figures are recorded for the uric acid content of the blood in gout which are open to question. It is possible by dietary restrictions in the purine-containing foods to lower the blood uric acid in gout, but this would scarcely account for the low results reported by some observers, notably some of those of Pratt, and Daniels and McCrudden. The latter investigators reported two cases of gout in women whose blood uric acids were quite normal. It appears rather strange that McClure and Pratt[21] should have thought it wise to omit all reference to work carried out with Benedict's modification of the uric acid method, when they admit that Folin had offered certain criticisms of his own method, their excuse being that the reported figures obtained with the modified method were too high to be compared with those obtained by the use of the original method.

From the normal variations of from 2 to 3 mg. to 100 c.c. of blood, the uric acid may increase to as much as from 4 to 9 mg. in gout, but it does not follow that these uric acid accumulations are infallible signs of gout, since, as noted above, similar uric acid figures may be found in nephritis. This point is well brought out in the table below taken from Fine. A very close

*The author is under obligation to Drs. Fine and Killian for many unpublished observations on uric acid recorded in this paper. He is also under obligation to Dr. Fine, now of the Calco Chemical Company for furnishing the tolysin (neocinchophen) and most of the cinchophen employed in the experiments reported in Table IV.

TABLE III

BLOOD PICTURES IN GOUT AND EARLY INTERSTITIAL NEPHRITIS*

CASE	DATE 1915-1916	AGE	SEX	DIAGNOSIS	URIC ACID, MG. PER 100 C.C.	UREA N, MG. PER 100 C.C.	CREATININE, MG. PER 100 C.C.	PHTHALEIN 2 HRS. PER CENT.	SYSTOLIC BLOOD PRESSURE	URINE	
										PROTEIN	CASTS
M. K.	9/ 3	49	♀	Typical gout.....	9.5	13	1.1	48	230	+	−
T. B.	10/ 5	57	♂		8.4	12	2.2	35	164	−	+
H. L.	3/24	51	♂		7.8	12	2.9	59	120	−	−
L. J.	10/ 6	43	♂		7.2	17	2.4	..	200	−	−
C. P.	10/ 6	45	♂		6.8	14	1.7	..	...	....	....
B. D.	3/31	25	♀	Miscellaneous cases	7.7	20	2.6	45	168	+	−
D. S.	6/ 7	56	♂	Slight edema..... Hypertension	6.7	19	2.5	26	185	−	+
E. V.	3/20	14	♀	Endocarditis	6.3	12	3.6	32	120	++	+
H. B.	3/28	55	♂	Showing some evidence of early interstitial nephritis	Incipient nephritis...	6.1	16	2.8	57	120	−
H. J.	3/23	50	♂		Diabetes	6.0	18	2.9	36	140	+
G. C.	3/14	40	♀		Incipient nephritis...	5.5	15	2.1	46	147	−
M. S.	3/22	46	♀		Gastritis	5.0	12	2.5	52	190	+

*Taken from Fine.[20]

scrutiny of Table III does disclose a tendency for the urea and creatinine concentrations of Group 2 to rise above those of Group 1, which are essentially normal, but the differences are slight. Myers and Fine[11] have stated, "The blood pictures in early interstitial nephritis and gout are strikingly similar, particularly as regards the increase in uric acid. In view of the other clinical symptoms in common, it would seem that this similarity must be more than accidental." Fine[20] has raised these questions: "1. Is gout merely a stage in the development of interstitial nephritis, whose further progress may be indefinitely delayed? 2. Is early interstitial nephritis merely potential gout, in which the clinical symptoms may or may not appear? 3. Is the uric acid retention of gout due to the specific condition, *gout*, or to a complicating early interstitial nephritis?" McClure[24] has studied the renal function in six cases of gout in which the clinical diagnosis of nephritis was not justifiable. By means of the phthalein test, nonprotein and urea nitrogen and two-hour renal test, some disturbance of renal function was demonstrated in each case, thus strengthening the general argument given above.

Practically, we may conclude that gout is almost invariably associated with an increased uric acid content of the blood and therefore a high blood uric acid may be of considerable diagnostic value in cases of gouty arthritis, in which tophi containing sodium urate are not already present. It should be borne in mind that cases of arthritis may occasionally show an increase in the uric acid, although this is generally associated with an increased urea and nonprotein nitrogen, thus indicating a complicating nephritis. Such cases of arthritis, however, are the exception rather than the rule. In ten typical cases of arthritis which have come under our observation during the past month the figures for the blood uric acid have ranged from 1.6 to 3.6 mg. to 100 c.c. and all but three cases showed under 3 mg.

INFLUENCE OF DIET AND DRUGS UPON THE BLOOD URIC ACID

That a purine-free diet will definitely, although not markedly lower the blood uric acid in gout is brought out by the observations on Case 12 in Table II, where in the course of about three weeks the uric acid fell from 7.8 to 6.4 mg., but again rose to 8.2 and 8.1 mg. upon the return to a purine diet. A much more pronounced lowering of the uric acid as the result of dietary restrictions may be noted in the first two cases of Table IV.

It has been recognized for some time that the administration of salicylates and cinchophen (2-phenyl-quinolin-4 carboxylic acid) resulted in an increased excretion of uric acid. Recently it has been shown by Folin and Lyman,[25] McLester,[15] Fine and Chace,[17] and Smith and Hawk[26] that the action of cinchophen was accompanied by a marked drop in the uric acid content of the blood and later the same was shown to be true of the salicylates by Fine and Chace[27] and Denis.[28] The action of phlorhizin and the methylated purines on the kidney is probably better known but scarcely as remarkable as that of the drugs mentioned. The action of phlorhizin holds little of clinical interest, and Christian and his coworkers[29] have recently thrown serious doubts on the therapeutic value of methylated purines.

It is now generally assumed that cinchophen and the salicylates induce an increased output of uric acid in the urine and a decreased concentration in the

TABLE IV

INFLUENCE OF DIET AND DRUGS UPON THE BLOOD URIC ACID

CASE	AGE	SEX	DATE	BLOOD ANALYSES MG. TO 100 C.C.			CLINICAL DIAGNOSIS, REMARKS
				Uric Acid	Urea N	Creatinine	
1. P. P.	28	♂	3/14/16	8.0	20	2.7	Gastric ulcer; diet until 3/17 contained broths, but later was purine-free.
			3/17/16	6.3	15	2.4	
			3/28/16	(3.7	20	...	
			4/ 4/16	(3.1	14	1.8	
2. M. S.	46	♀	2/23/16	6.1	12	2.6	Arteriosclerosis, periodic vomiting; diet purine-free from 2/29 until after 3/16; 60 grains of sodium salicylate 3/27 to 4/2, then purine-free diet.
			3/ 7/16	(3.1	12	2.9	
			3/16/16	(3.0	12	2.5	
			3/23/16	5.0	12	2.5	
			3/31/16	(1.7	13	3.4	
			4/ 7/16	2.5	..	...	
			4/18/16	5.0	12	2.3	
3. T. G.	46	♂	6/27/16	6.0	17	2.6	Chronic duodenal ulcer, syphilis; purine-free diet 3 days previous to 1st analysis; 30 grains of a combination of Na salicylate and cinchophen daily for 4 days preceding 2nd analysis.
			7/ 1/16	(3.2	18	1.8	
4. M. G.	58	♂	3/26/20	6.0	29	1.5	Chronic interstitial nephritis, arteriosclerosis; 50 grains of tolysin given 3/28 to 4/3.
			3/28/20	5.2	29	2.4	
			3/31/20	(trace	16	2.0	
			4/ 5/20	3.0	24	2.2	
			4/12/20	3.5	18	3.0	
			4/13/20	3.9	15	2.4	
5. M. D.	53	♀	3/16/20	2.8	26	2.6	Neurasthenia, visceroptosis; vegetable diet; tolysin 50 grains daily 3/19-22; cinchophen 50 grains daily 4/9-12.
			3/18/20	2.8	22	2.6	
			3/22/20	(trace	13	2.7	
			3/25/20	2.6	20	2.4	
			3/29/20	2.6	19	2.2	
			4/ 9/20	2.8	16	2.4	
			4/12/20	(0.7	13	2.6	
			4/15/20	2.0	17	2.4	
6. G. McK.	21	♀	12/ 4/19	9.3	48	13.4	Chronic interstitial nephritis; patient died 12/30; 25 grains cinchophen daily 12/5-8 with lemonade diet; low protein diet with 25 grains of a mixture of cinchophen and theobromine daily 12/9-11.
			12/ 5/19	10.4	42	16.8	
			12/ 6/19	(5.1	52	12.7	
			12/ 7/19	(8.3	45	15.0	
			12/ 8/19	(8.1	48	14.3	
			12/ 9/19	(5.4	55	12.3	
			12/11/19	(7.5	57	12.1	
			12/13/19	6.1	53	13.0	
7. S. H.	37	♂	5/10/15	7.7	76	10.9	Chronic interstitial nephritis; 45 grains cinchophen on 5/12 and 13, 60 grains on 5/14; patient died 6/28.
			5/15/15	(8.0	64	9.6	
			5/21/15	7.8	53	8.0	

blood (and tissues) by endowing the renal cells with an increased power for eliminating uric acid. The effect of these drugs begins to be manifest nearly as soon as absorption of the drug has taken place and exerts its maximum influence in about one day.

Both of these drugs have marked analgesic effects, as well as that of stimulating the excretion of uric acid, and it would appear difficult to decide as to which effect was the more valuable in conditions with high blood uric acids, such as gout. Although the action of these drugs is very similar, cinchophen

and neocinchophen, its methyl derivative, have an advantage in that they appear to be free from the toxic influences on the kidney inherent in the salicylates. Tolysin (neocinchophen) is tasteless and is better tolerated by some patients than cinchophen.

The influence of these drugs upon the blood uric acid is shown in Table IV. Case 2 illustrates the effect of sodium salicylate and Case 3 a combination of this drug with cinchophen. In Cases 4 and 5 tolysin was employed, Case 4 showing a high and Case 5 a normal initial uric acid. The control urea nitrogen in both cases was somewhat above normal. In both cases the uric acid was reduced to a quantity too small to estimate, and there was likewise a definite influence on the urea.[30] Cinchophen was subsequently employed in this case with a somewhat less pronounced effect. Fine and Chace[31] have pointed out that in the last stages of interstitial nephritis cinchophen has little influence on the excretion of the uric acid, indicating that the renal cells can no longer be stimulated to increased activity. Cases 6 and 7 illustrate this point, no change in the uric acid being noted in the last of these two cases, even after large doses of cinchophen. We are studying further the comparative action of various drugs in this connection.

ESTIMATION OF URIC ACID

In 1912 Folin and Macallum[32] called attention to the possibilities of the use of phosphotungstic acid in the colorimetric estimation of uric acid, and later in the same year Folin and Denis[2] gave their first description of this estimation as applied to blood. As already pointed out, the results which they first obtained with this method clearly demonstrated the increase in the blood uric acid in gout and lead poisoning[6] and in nephritis.[7] In carrying out these determinations the procedure was to coagulate the blood protein with weak (0.01 N) acetic acid after a 1 to 6 dilution with this solution, filter, wash, evaporate to a very small volume and then precipitate the uric acid with silver lactate, magnesia mixture and ammonia in a small centrifuge tube. (Owing to the presence of phenols in blood it is necessary to separate the uric acid from these color reacting substances.) This was then thrown down in the centrifuge and the precipitate decomposed with hydrogen sulphide. After removal of the excess of hydrogen sulphide, the color reaction was then developed with the special phosphotungstic acid reagent and sodium carbonate.

It could hardly have been expected that such a new method would be perfectly satisfactory in every detail, and in 1915 Benedict[33] introduced certain modifications which did much to increase the accuracy and simplicity of the method. Possibly the greatest source of error was in the decomposition of the silver precipitate with hydrogen sulphide, since low results were obtained, unless considerable care was taken in breaking up the precipitate. For this purpose Benedict employed potassium cyanide, which has proved to be very satisfactory. Other improvements suggested by Benedict were a more dependable standard uric acid solution, the combined use of the silver lactate, magnesia mixture and ammonia into a single reagent and the use of colloidal iron to remove the last trace of protein after coagulation. For this purpose we had been employing alumina cream, and continued to use this in preference to the colloidal iron. Otherwise we at once adopted the suggestions of Benedict. Although this method[11] was

somewhat tedious, various checks, which we have made from time to time during the past five years, have led us to believe that it gives perfectly reliable results. A year ago Folin and Wu,[34] in connection with their system of blood analysis, described a method of estimating uric acid directly in the protein-free filtrate obtained after their tungstic acid precipitation without evaporation of the filtrate. Such a procedure obviously greatly simplified the technic of this estimation. In order to secure a permanent standard Folin and Wu have made use of sodium sulphite, which prevents oxidation of the uric acid. As they have noted, the use of this reagent cuts down the peculiar intensification of the blue color obtained by the use of cyanide. They state: "Opinions will doubtless differ as to whether this is an advantage or disadvantage." We are inclined to take the latter view.

In the method described below we have made use of Folin and Wu's new protein precipitant and their lactic acid-silver lactate reagent to precipitate the uric acid, but after the decomposition of the precipitate we still adhere to the suggestions of Benedict. The method as described yields essentially the same results as those obtained with the technic we formerly employed. Fairly satisfactory recoveries of added uric acid may be obtained, provided the proper strength of sulphuric acid has been employed. As Folin and Wu have pointed out, an excess of acid will bring about a precipitation of the uric acid.

Method.—To 5 c.c. of well-mixed oxalated blood in a 100 c.c. Erlenmeyer flask (or 100 c.c. glass stoppered cylinder), add 35 c.c. of water (7 volumes), 5 c.c. of 10 per cent sodium tungstate and then 5 c.c. of exactly 2/3 N sulphuric acid while rotating the flask. Shake thoroughly. (In practice we frequently employ 7 c.c. of blood and in this way rather quickly secure 40 c.c. of filtrate, the equivalent of 4 c.c. of blood. Perfectly satisfactory results may be obtained with 2.5 or 3 c.c., however.) When the blood is properly coagulated, the color of the coagulum turns from pink to brown. If this change does not occur, the coagulation is incomplete, due probably to too much oxalate. In such a case, 5 per cent sulphuric acid may be added a drop at a time, shaking between each addition, until the coagulation is complete. Folin and Wu *caution against* an *excess* of sulphuric acid, since this apparently brings about a precipitation of the uric acid. If the mixture is now carefully poured upon the double portion of a filter just large enough to hold the mixture, the filtrate will probably come through perfectly clear; if not, the first portion may be returned to the filter. To prevent evaporation, a watch glass may be placed over the top of the funnel. It is convenient to filter into a 50 c.c. graduated cylinder.

When the filtration is complete, record volume (ordinarily between 25 and 30 c.c.), and pour contents of cylinder into a 50 c.c. centrifuge tube. Add 5 c.c. of 5 per cent silver lactate solution in 5 per cent lactic acid. After stirring, centrifuge. Pour off and discard supernatant fluid. To the precipitate add about 3 c.c. of 10 per cent sodium chloride in 0.1 N hydrochloric acid* and stir with glass rod. Then add 3 to 4 c.c. of water, 1 drop of 5 per cent potassium cyanide, stir again and centrifuge. This treatment sets free the uric acid from the precipitate. Pour the supernatant fluid into an accurately graduated 25 c.c. cylinder.

Into a similar 25 c.c. cylinder introduce with an Ostwald-Folin pipette 1 c.c. of standard uric acid solution** (contains 0.2 mg. uric acid). Add 4 c.c. of the acidified

*The 10 per cent solution of sodium chloride in 0.1 N hydrochloric acid may be prepared by adding 1 c.c. of concentrated hydrochloric acid to 100 c.c. of 10 per cent chloride solution.

**Benedict's Standard Uric Acid Solution.—This is prepared as follows: Dissolve 4.5 gm. pure crystalline hydrogen disodium phosphate and 0.5 gm. dihydrogen sodium phosphate in 200 to 300 c.c. hot water. Filter and make up to about 250 c.c. with hot water. Pour this warm, clear solution on 100 mg. uric acid suspended in a few c.c. of water in 500 c.c. volumetric flask. Agitate until completely dissolved. Add at once exactly 0.7 c.c. glacial acetic acid, make up to 500 c.c., mix and add 5 c.c. chloroform. One c.c. of this solution contains 0.2 mg. uric acid. This solution should be freshly prepared every 2 months.

sodium chloride solution and sufficient water to make the volume essentially the same as the unknown. Then add 1 drop of 5 per cent potassium cyanide.

To the standard now add 1 c.c. of the uric acid reagent* and 0.5 c.c. to the unknown, and then 5 to 6 and 3 to 4 c.c. of saturated sodium carbonate respectively. Allow the color to develop for about 5 minutes, then dilute the standard to 20 or 25 c.c., and the unknown, if darker than the standard, to a similar depth of color.

With this technic, fairly deep blue colors are obtained even with normal blood, colors that can readily be matched in the colorimeter. The slight cloud that sometimes appears may easily be separated in the centrifuge. For the calculation the following formula may be used: $\frac{S}{R} \times \frac{X}{25} \times \frac{0.2 \times 100}{B}$ = mg. uric acid to 100 c.c. of blood, in which S represents the depth of the standard (15 mm.), R the reading of the unknown, X the dilution of the unknown, 0.2 the strength of the standard in mg., and B the number of c.c. of blood to which the filtrate employed was equivalent.

ESTIMATION OF URIC ACID WITH THE TEST TUBE COLORIMETER

The technic described above readily lends itself to use with the test tube colorimeter.

Method.—Three c.c. of the well mixed oxalated blood are pipetted into a large test tube or small flask and 21 c.c. of water added. The precipitation of the proteins is now carried out according to the procedure of Folin and Wu, as described above, 3 c.c. each of the sodium tungstate and 2/3 N sulphuric acid being added.

In case it is necessary to add extra sulphuric acid, this should be done carefully as an excess may lead to a precipitation of the uric acid.

To 15 c.c. of the filtrate in a 20 c.c. centrifuge tube† add 2 c.c. of 5 per cent silver lactate in 5 per cent lactic acid. After stirring, centrifuge. Pour off and discard the supernatant fluid. To the precipitate add about 1.5 c.c. of 10 per cent sodium chloride in 0.1 N hydrochloric acid and stir with glass rod. Then add 3 to 4 c.c. of water, stir again and centrifuge. This treatment sets free the uric acid from the precipitate. Pour the clear supernatant fluid into the right hand tube of the colorimeter. Add 1 drop of 2.5 per cent potassium cyanide.

Into the left hand tube of the instrument introduce 1 c.c. of the standard uric acid solution (diluted 1-5, contains 0.04 mg. uric acid). Add 1.5 c.c. of the 10 per cent sodium chloride in 0.1 N hydrochloric acid and sufficient water to make the volume essentially the same as in the unknown. Then add 1 drop of 2.5 per cent potassium cyanide.

To both tubes now add 0.3 c.c. of the uric acid reagent and 2 c.c. of saturated sodium carbonate solution. Allow the color to develop for 3 to 5 minutes, and then dilute the standard to 10 c.c. (20 c.c. if the unknown shows a weak color development) and the unknown to the same intensity of color as the standard, adding the water a drop or two at a time just before the end point in matching colors is reached.

To calculate the mg. of uric acid per 100 c.c. of blood, the following formula may be used, in which S represents the strength of the standard (0.2 or 0.4 mg. to 10 c.c., depending on the dilution), D the dilution in c.c. of unknown required to match the standard, and B the amount of blood employed in c.c.: $\frac{}{B}$ = mg. uric acid per 100 c.c. of blood. If, for example, 15 c.c. of filtrate are used, the equivalent of 1.5 c.c. of blood with a

*Benedict's Modification of Folin-Denis Uric Acid Reagent.—Prepared by boiling 100 gm. of sodium tungstate, 20 c.c. concentrated hydrochloric acid and 30 c.c. 85 per cent phosphoric acid in 750 c.c. distilled water for 2 hours, preferably under a reflux condenser, and then making up to 1000 c.c. with water.

†Cylindrical trunnion cups may be substituted for the conical cups of the ordinary centrifuge. In these, test tubes or special 20 c.c. centrifuge tubes may be employed, and the cups work quite as well for the 15 c.c. conical centrifuge tubes.

standard containing 0.4 mg. diluted to 10 c.c., and the dilution of the unknown required to match the standard is to 17.1 c.c., the formula will work out as follows:

$$\frac{0.4 \times 17.1}{1.5} = 4.6 \text{ mg. to } 100 \text{ c.c.}$$

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BACTERIOLOGIC DATA ON THE EPIDEMIOLOGY OF RESPIRATORY DISEASES IN THE ARMY*

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ALTHOUGH many investigators have tried to determine the exact route taken by the specific microorganisms of respiratory diseases in passing from one person to another, opinion on this subject is still divided into two more or less opposing hypotheses. According to one, the microorganisms are chiefly air-borne and are inhaled, either at some distance from their source, or usually at short range in the droplets of coughs and sneezes. According to the other theory, the air is practically free of pathogenic microorganisms which settle out quickly or die by drying. The organisms are believed to travel in infected discharges on some intermediate object such as the hands, cups, etc., and then back to the mouth. For brevity, one may be called the direct and the other the indirect method.

I. THE INDIRECT METHOD

The experiences of the war reawakened an interest in the general problem as it affects soldiers, which was brought to a focus by the papers of Lynch and Cumming.[1] These observers presented data, largely statistical, to show that the principal route of travel is "mouth to hand and hand to mouth" through the medium of the dish water in which mess kits are washed. Under direction of Colonel F. F. Russell, M.C., in charge of the Division of Laboratories and Infectious Diseases of the Surgeon General's Office, some work was planned on this subject along with other problems of respiratory diseases. Major Oscar Teague was selected to obtain some actual bacteriologic evidence on the general problem. He made a large number of observations at Camp Meade, Maryland, in December, 1918, and January, February and March, 1919, and made an extensive detailed report to the Surgeon General, which covered a number of points relating to the spread of respiratory diseases. Most of the results of the field work recorded in this paper have been extracted from Major Teague's noteworthy report. The writer is responsible for the general discussion, the work on the antiseptic action of soap and for the conclusions. While the data collected are not considered as at all final or entirely conclusive, they are believed to be of sufficient value to warrant publication, and so far as they go, they support the air-borne theory.

ORGANISMS USED—HEMOLYTIC STREPTOCOCCI

The first problem was to find a suitable organism to work with. The search for specific microorganisms with the possible exception of hemolytic streptococci was not considered feasible. It was at first proposed to use some easily recognizable nonpathogenic organism which could be inoculated in the nose and throat

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and then followed on its travels. B. prodigiosus has been considerably used along these lines, but it was soon found that this organism does not parasitize in the nose and throat and disappears a few hours after inoculation. A similar observation has been made by Bloomfield[2] in regard to a pigmented coccus. B. prodigiosus was also found to die on the hands in 10 to 20 minutes. Some further preliminary work was also done with Deneke's vibrio, a black mould, a red coccus, and an orange yeast, but none of these organisms were suitable. Finally, on account of the presence of a large number of throat carriers of hemolytic streptococci and the known viability of these organisms, it was decided to use them. Large numbers of streptococcus carriers were produced in camps in some way, and it was thought that a search for streptococci might throw some light on their epidemiology. The streptococci would also in a measure serve as indicators of the presence of sputum and salivary contamination in the same way that bacilli of the colon group serve as indicators of fecal contamination. Streptococci are not as suitable for this purpose as colon bacilli on account of the difficulty of enrichment, but they seemed the most suitable organisms available.

Some preliminary work was done to determine the length of time streptococci would persist on the hands. A specimen of sputum from a patient who was critically ill of pneumonia, freshly expectorated at 1:35 P.M. was collected in a sterile Petri dish. At 2:15 P.M. a bit of sputum (¼ c.c. to ½ c.c.) was rubbed into a piece of sterile gauze about the size of a handkerchief and the portion of the gauze thus moistened was rubbed over the palmar surface of the tips of all the fingers of O. T. The fingers were then thoroughly wiped with another piece of dry, sterile gauze. The amount of sputum upon the fingers must have been extremely small. At intervals one finger tip after the other was streaked over half the surface of a blood agar plate; the other half was streaked with a platinum loop. Examination of the plates gave the following results:

TABLE I

		NUMBER OF COLONIES OF HEMOLYTIC STREPTOCOCCI
Right hand—5th finger	At once	240
4th finger	½ hour	300
3rd finger	1 hour	187
2nd finger	1¼ hours	19
Left hand—5th finger	3 hours	15
4th finger	4 hours	27
3rd finger	5 hours	44
2nd finger	6 hours	59
1st finger	8 hours	13

These results show that a slight contamination of the hands is detectable for a number of hours. If natural contamination occurs to any great extent, it too should be detectable for some time. A similar experiment made with influenza bacilli in sputum showed that these organisms died in about three minutes. The criterion for hemolytic streptococci was the production of a hemolyzing colony which when picked to broth showed Gram-positive cocci in chains.

The work was carried out on troops of Sanitary Trains at Camp Meade. The throats and tonsils of 318 men were cultured for hemolytic streptococci, and

159, or 50 per cent, were found positive in varying degrees. An effort was then made to trace these organisms in carriers' hands and in their environment. As is well recognized, the crypts of the tonsils are the principal homes of hemolytic streptococci in carriers.[3] The saliva is infected from this source in varying degrees, according to circumstances. In one series of nine carriers the saliva was positive in only three. In another nine cases the saliva was positive in all.

EXAMINATION OF FINGERS

As a control, nine men with positive throat cultures rubbed the index finger back and forth on their lips and then streaked it on a part of a blood agar plate which was more fully spread with a platinum loop. Six of these plates showed colonies ranging from ten to many.

The fingers of carriers were examined in the middle of the morning, (1) by rubbing the index finger of the right hand over part of an agar plate and then spreading by a loop; (2) by washing the fingers in a small amount of salt solution and plating a few drops on agar plates. Out of 159 carriers examined in this way, 27, or 16.9 per cent were positive. More than half of these positive plates showed only a single colony and only five showed more than five colonies.

EXAMINATION OF DISH WATER

The next step was to examine dish water. Dish water was collected from 24 messes after the dinner dishes had been washed. The number of men in these messes ran from 30 to 300. The temperature was not sufficient to kill streptococci—averaging about 38°. One large drop was spread on a blood agar plate. None of these specimens showed any hemolytic streptococci. In eight messes lukewarm water without soap was used by request, and in one of these one colony of streptococci was found and in another two colonies. The antiseptic action of soap will be considered later. Scrapings from dish mop handles showed no streptococci.

HAND SHAKING

Several experiments were made to obtain evidence on the related problem of the importance of hand shaking in the spread of streptococci. (1) A bit of sputum of patient Reid, who was known to harbor hemolytic streptococci in his throat, was taken up on a piece of sterile gauze about the size of a handkerchief and rubbed well into the same. The moistened portion of the gauze was rubbed over the palmar surface of the fingers of O. T. and the fingers were immediately wiped dry with a fresh piece of sterile gauze. After an interval of 45 minutes O. T. shook hands once with McC., who then rubbed the side of his hand over the surface of a blood agar plate. A small sterile cotton swab was moistened with sterile saline, rubbed over the portion of McC.'s hand that came in contact with the fingers of O. T., and streaked over a blood agar plate.

	NUMBER OF COLONIES OF HEMOLYTIC STREPTOCOCCUS
Fingers of O. T. (control)	13
Side of hand McC. No. 1.	0
Side of hand McC. No. 2.	0
Swab	0

(2) A bit of the mixed sputum of Doyle and Clark was taken up on a piece of sterile gauze. The moistened portion of the gauze was rubbed over the palmar surfaces of the fingers of O. T. who twenty minutes later shook hands with McC.

	NUMBER OF COLONIES OF HEMOLYTIC STREPTOCOCCUS
Fingers of O. T. (control)	Numerous
Side of McC.'s hand No. 1.	1
Side of McC.'s hand No. 2.	2
Swab	0

(3) A bit of sputum of patient Meneice, convalescent from streptococcus pneumonia, was taken up on sterile gauze and the moistened portion of the gauze was rubbed over the palmar surface of the fingers of the right hand of O. T. Thirty minutes later O. T. shook hands once with McC.

	NUMBER OF COLONIES OF HEMOLYTIC STREPTOCOCCUS
Fingers of O. T. (control)	about 400
McC. side of hand No. 1.	1
McC. side of hand No. 2.	8
Swab	1

It will be seen from these experiments that few streptococci are transferred in handshaking, and that they reach a part of the hand which does not usually come in contact with the mouth.

POSSIBLE TRANSFER TO THE MOUTH

The final possible step of infecting the mouth from the hands was tested in the following way: Three men on duty in the laboratory of the Walter Reed Hospital volunteered for this experiment. Cultures from their nasopharynx, tonsils and nostrils were negative for hemolytic streptococci on three successive days before the experiment. The tonsils of the two men known to harbor hemolytic streptococci were swabbed and Majors Teague and Simmons and the three volunteers in turn rubbed the index finger over the swabs and immediately afterwards passed the same finger three or four times across the closed lips. The lips (or faces) were not washed during the next five or six hours. The nasopharynx, tonsils and nostrils of the volunteers were cultured almost daily for the next two weeks on blood agar plates; the cultures were negative for hemolytic streptococci.

It will thus be seen that so far as streptococcus carriers are concerned, according to this evidence, they must have been produced in some other way than by "mouth to hand and hand to mouth" method of transferring streptococci through the medium of dish water. Although the fingers can be intentionally contaminated with streptococci by saliva and although the organisms live on the fingers for hours, under ordinary circumstances few streptococci were found on the fingers, and none in the dish water, except when no soap was used. Furthermore, infection of the mouth from infected fingers is not as easy as might be supposed.

As the hand is more generally recognized as a method of spread of causes of

intestinal diseases, comparative observations were made, using the colon bacillus group as an indicator of fecal contamination. In order to determine the amount of colon bacillus contamination of the hands of soldiers, 147 men of five field organizations were examined. The tips of the five fingers of the right hand were rubbed together in 5 c.c. of sterile salt solution in a sterile Petri dish, and 3 c.c. was planted in lactose broth fermentation tubes. The work was done indoors about the middle of the morning. After incubation for 48 hours over 20 per cent of gas was considered a presumptive test for the colon group. In a few instances complete identification of the colon group was made. Out of 143 men examined, 42, or 29.4 per cent were found positive. It was thus shown that nearly one-third of these men's fingers carried colon bacilli, presumably of intestinal origin.

The contamination of dish water was then looked into, with the following results:

TABLE II

	DISH WATER USED BY		TEMP.	COLON BACILLI IN 1 C.C.	FOUR DROPS	1/100 C.C.
1.	210 men		31	–	–	–
2.	250 "	Soapy	33	–	–	–
		Rinse	37	–	–	–
3.	200 "	Soapy	36	–	–	–
		Rinse	37	–	–	–
4.	125 "	Soapy	35	+	+	–
		Rinse	37	+	+	+
5.	250 "	Soapy	31	+	–	–
6.	91 "	Soapy	31	+	–	–
7.	140 "	Soapy	38	+	+	+
		Rinse	41	+	+	+

Table II shows the variation dependent on use of different quantities of water, but even in 1/100 c.c. colon bacilli were present in two out of eleven specimens. In order to compare the number of colon bacilli with that of streptococci more exactly, the following observations were made. The same amount of dish water, four drops, was plated on blood agar and put into lactose fermentation tubes with the results shown in Table III.

TABLE III

	DISH WATER USED BY		TEMP.	PLAIN AGAR COLONIES PER C.C.	BLOOD AGAR OTHER COLONIES	H. S.	LACTOSE FERMENTATION TUBE COLON GROUP
1.	210 men	Dish water	31	550	36	0	0
2.	250 "	Wash "	33	3500	many	0	0
		Rinse "	37	40	4	0	0
3.	125 "	Wash "	35	1550	176	0	+
		Rinse "	37	2000	200	0	+
4.	250 "	Wash "	31	6300	338	0	0
5.	91 "	Wash "	31	290	23	0	0
6.	200 "	Wash "	31	900	199	0	0
		Rinse "	37	1500	113	0	0
7.	140 "	Wash "	38	3290	380	0	+
		Rinse "	41	720	290	0	+

In this work no streptococci were found in an amount which gave 4 out of 11 tests positive for colon bacillus.

These data on the colon bacilli show that bacteriologic evidence can easily be obtained to support the hypothesis that the fingers are concerned in the spread of intestinal diseases. Colon bacilli were found on the fingers and in dish water under conditions which showed no streptococci. The inference is that these two groups of organisms and the diseases for which they stand are spread in different ways.

II. The Antiseptic Action of Soap in Dish Water

The antiseptic action of soap in dish water has a direct bearing on the results given above. With the assistance of Captain Stimmel, S. C., a number of observations have been made on various soaps and on dish waters; the effect of different hydrogen-ion concentrations has been considered. Several of the soaps were obtained through the kindness of Mr. Powell of Armour Soap Works. The average strength of soap solution in army messes seems to be about 0.5 per cent, and this strength has been chiefly used in tap water; the organisms were added to a solution of this strength and 0.1 c.c. was spread on blood agar plates after various intervals. Table IV gives some of the typical results:

Table IV

SOAP	TIME OF EXPOSURE IN MINUTES AND RESULTS								
	Organism	½	1	2	5	10	Control	P H	Temperature
Sodium Oleate (Armour)	B. Influenzæ	–	–	–	–	–	In*	8.7	Room
	Pneumococcus	–	–	–	–	–	In	"	"
	Streptococcus pyogenes	–	–	–	–	–	In	"	"
	S. Aureus	In	In	In	In	In	In	"	"
	B. Typhosus	In	In	In	In	In	In	"	"
Sodium Stearate (Merck)	B. Influenzæ	In	In	In	–	–	In	8.2	"
	Pneumococcus	In	In	In	In	–	In		
	Streptococcus pyogenes	In	In	In	In	In	In		
	S. Aureus	In	In	In	In	In	In		
	B. Typhosus	In	In	In	In	In	In		
Sodium Resonate (Armour)	B. Influenzæ	–	–	–	–	–	In	8.2	"
	Pneumococcus	–	–	–	–	–	In		
	Streptococcus pyogenes	–	–	–	–	–	In		
	S. Aureus	+	+	+	+	+	In		
	B. Typhosus	+	+	+	+	+	In		
Brown Soap (Camp Meade)	B. Influenzæ	–	–	–	–	–	In	8.7+	"
	Pneumococcus	–	–	–	–	–	In		
	Streptococcus pyogenes	++	+	15	–	–	In		
	S. Aureus	In	In	In	In	In	In		
	B. Typhosus	In	In	In	In	In	In		

*In = Innumerable.

The difference in effect of sodium oleate and sodium stearate is brought out, and more particularly the effect of ordinary brown soap, largely sodium resonate, such as is used in dish water in army messes. This soap reduced the number of streptococci from innumerable to 15 in two minutes. Hence, if any streptococci did get from the hands into the dish water they would have small chance to spread further, provided the dish water was soapy. On the other hand, none of the

soaps tested killed the typhoid bacillus in 10 minutes. At low temperature, therefore, there is no barrier to the spread of intestinal organisms. The streptococci were killed whether the temperature was 38° or room temperature.

The point was raised by a sanitary inspector whether in a dirty dish water full of grease the antiseptic action might not be lost. To test this idea some boiled mutton fat was added to the soap, but no change occurred, either in reaction or in antiseptic effect. However, it was found that if the reaction of these soaps was changed from about 8.5 to 7 by the addition HCl, their antiseptic action was lost. This result is apparently due to precipitation of the soap from solution. Free alkali apparently plays no part in the antiseptic action as a NaOH solution of P_H 9.6 has no antiseptic effect. But an alkaline solution is necessary and the reading of the reaction of dish waters might be a valuable index for their suitability, as there is apparently enough acid in some dirty dishes to change the reaction and destroy the antiseptic effect. This is seen in the examination of two dish waters as shown in Table V.

TABLE V

		TIME OF EXPOSURE IN MINUTES AND RESULTS						PH	TEMP.
SOLUTION	ORGANISM	1/2	1	3	5	10	CONTROL		ROOM
Dish Water 242 F.H. Before use	B. Influenzæ	20 colonies	–	–	–	–	In	8.7	
	Pneumococcus	–	–	–	–	–	In		
	Streptococcus pyogenes	18 col.	–	–	–	–	In		
	S. Aureus	In	In	In	In	In	In		
	B. Typhosus	In	In	In	+	+	In		
244 F.H. After use	B. Influenzæ	In	In	In	In	In	In	5.8	
	Pneumococcus	In	In	In	In	In	In		
	Streptococcus pyogenes	In	In	In	+	+	In		
	S. Aureus	In	In	In	In	In	In		
	B. Typhosus	In	In	In	In	In	In		

In the second case the reaction was 5.8 and no antiseptic action occurred. This was an especially dirty water which had been used by a large number of men.

It is well known that certain pure soaps are strongly antiseptic, while others are more or less inert. In general, as Lamar[4] has pointed out, chemical unsaturation, hemolytic activities, and antiseptic action go together. For example, sodium oleate, which in a low dilution kills streptococci rapidly, is hemolytic and chemically unsaturated. On the other hand, sodium stearate is made of a saturated fatty acid and is nonhemolytic and nonantiseptic in similar dilutions.

In addition certain soaps have selective action on certain kinds of bacteria and the possibility suggests itself of special soaps for killing special organisms or of a synthetic soap which will kill a number of different kinds of organisms. Commercial soaps are usually a more or less chance mixture of salts of different fatty acids, or other saponifiable substance, and are not built up from this point of view. Sodium oleate has been used in plates by Avery[5] to inhibit the action of other organisms of the nose and throat and to aid the growth of influenza bacilli. This action is definite in plates, but in solution the oleate seems to kill the influenza bacillus as well as the other organisms. Reasoner[6] has shown that soap solution from Ivory soap and shaving creams have a very rapid solvent effect on

T. pallidum. In case pure soaps are used, sodium oleate shows this effect to a marked degree, but with sodium stearate, very little effect even on motility can be observed after a number of minutes.

As bearing on the problem in hand, it will be seen that the streptococcus is quickly destroyed in the average soapy dish water at ordinary temperature, and it does not seem possible that the few that reach the dish water can survive to pass on to another person. The practical point emerges, however, that the reaction of the dish water should be kept at about a P_H of 8 or over, and that an acid dish water has no antiseptic effect. On the other hand, the ordinary dish washing in the army seems to offer no barrier to the spread of intestinal organisms, and a hot or boiling water is necessary to prevent the possible spread of these organisms.

III. The Direct Method

As no bacteriologic data in support of the indirect method of spread of streptococci could be obtained, efforts were made to determine what evidence for the direct method could be obtained.

The length of time that droplets remain suspended in the air

Two men with inflamed throats which culture showed contained numerous hemolytic streptococci went into a quiet room and sprayed their saliva in all directions through partly closed lips for five minutes. They then left the room and a masked observer (T) exposed blood agar plates in sets of three on the floor in the middle of the floor for various periods as given in Table VI.

Table VI

Length of time after spraying	Time of exposure in minutes	Total colonies	Number of H. S.
15 min.	15	740	10
30 "	30	1730	8
45 "	30	420	7
60 "	30	310	3
1¼ hrs.	30	180	2
1½ "	30	48	0
2 "	60	130	2
2½ "	30	58	6
3 "	60	84	4
4 "	60	83	5

Thus at the end of four hours streptococci were still settling out of the air. In order to investigate actual conditions of the air, a ward was selected in which there were 20-30 patients, of whom 75 per cent were streptococcus carriers, or cases such as tonsillitis. After the patients had retired, blood agar plates were exposed for three hours on chairs at the foot of the beds. On twelve nights a total of 155 plates were exposed. Nineteen of these, or 12 per cent, were positive for hemolytic streptococci. Since one streptococcus colony was present for every 387 square inches of surface of plates exposed, and the floor space of the ward was about 162 square meters, it follows that about 4000 streptococci settled out of the air in three hours.

A number of other experiments were carried out on the relative size of droplets and other related subjects which will appear elsewhere.[8] As bearing on

the so-called successful treatment of contagious diseases in the same ward without cross infection, the point is raised whether the patients are not usually received at hospitals after the very early stages which are probably the most contagious.

DISCUSSION

The interpretation which the writer puts on these data is that streptococcus carriers are not produced by contaminating their mouths with supposedly streptococcus laden dish water, but probably by inhalation. The evidence given above certainly favors this conclusion and if streptococci can be taken as indicators of respiratory organisms in general, the same conclusion holds good for them.

There is of course a large literature on this subject. In place of reviewing this literature and attempting to evaluate it, the writer believes it is more advisable at present to make fresh observations at different angles and to record them for what they are worth.

These data are subject to several limitations. The number of streptococci in carriers may be much less than the number of organisms in the watery discharges of the early stages of respiratory diseases. However, the results would still hold good for streptococcus carriers. Again in the comparison of streptococci and colon bacilli, it is to be remembered that only 50 per cent of the men carried streptococci, some in small numbers, while 100 per cent of persons carry large numbers of colon bacilli. On the other hand, the chances of hand contamination with streptococci are more frequent than with colon bacilli.

More observations can be made to advantage along the same lines and more attention could be paid to the possibility of the presence of agricultural colon bacilli and of streptococci from the stools or air, but it is not believed that more detailed work will materially alter the results.

In regard to the actual program proposed of using boiling wash and rinse water, while it is not believed that this measure will reduce the incidence of respiratory diseases, this work shows the desirability of this measure as a preventative of the spread of intestinal diseases. The necessity for the presence of a sufficient amount of good soap is also emphasized.

If the specific causes of respiratory diseases are spread principally by inhalation, more constant attention must be paid to sanitation (ventilation) and personal hygiene, and the route of spread can frequently be broken. However, in the army, there is a point beyond which such measures can not go. There are times when soldiers must train and fight in common, in spite of the presence of pathogenic organisms in the air. A certain amount of sickness and death from this source is to be counted on, unless the troops have some immunity from previous exposure or unless they can be immunized with specific vaccines.

SUMMARY

1. Hemolytic streptococci were used as test organisms in collecting data on the possible routes of spread of the specific causes of respiratory diseases.

2. No evidence was found to support the theory that these organisms spread through dish water.

(a) Fingers of only 17 per cent of carriers showed streptococci, and only in small numbers.

(b) Dish water showed no streptococci except when no soap was used.

(c) Infection of the mouth did not occur when streptococci were smeared on the lips.

(d) Soapy dish water is antiseptic for streptococci if of proper reaction and made with proper soap.

3. Evidence was found that intestinal organisms can spread through dish water.

(a) Colon bacilli were found on hands of nearly one-third of troops.

(b) They were found in dish water down to 1/100 of a c.c. in some cases.

(c) Soapy dish water has no antiseptic action on colon bacilli.

4. Evidence was also found to support the inhalation theory.

(a) Droplets with streptococci remain suspended for several hours.

(b) The air of streptococcus wards contains streptococci for several hours after men have retired.

5. The use of boiling dish and rinse water is indicated not to prevent spread of respiratory diseases, but to prevent spread of intestinal diseases.

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CHRONIC POISONING FROM HYDROCYANIC ACID

By C. I. Reed, A.B., Lawrence, Kansas

A SURVEY of the literature on cyanide poisoning reveals a considerable difference of opinion as to the existence of chronic symptoms in the event of recovery. There has existed a belief that if cyanide poisoning does not produce death within a few hours, there is eventual recovery without any chronic symptoms. It is the purpose of this paper to present a few experimental results on this point.

The greater majority of reports of cyanide poisoning credit patients with complete recovery with no chronic symptoms. Cohen[1] reports a severe case of accidental poisoning from potassium cyanide with complete recovery in a few hours with no chronic symptoms. Wilkes[2] describes the case of a druggist who absorbed hydrocyanic acid through a cut in the thumb, and as a result suffered very severe symptoms for a time but had completely recovered by the next day and subsequently showed no chronic symptoms. Numerous other reports follow a similar history.

On the other hand, Souwers[3] reports having examined a photographer who repeatedly was exposed to a solution of potassium cyanide in the course of his work and who exhibited listlessness, sleeplessness, lumbar pain, loss of appetite, nausea, constipation, chills, dyspnea and slowed pulse. These symptoms abated in a few days after ceasing work but returned on resumption of his occupation. Boddaert[4] observed a man poisoned by habitually eating bread rolls containing extract of bitter almonds, and after some weeks exhibited general lassitude, mental depression, muscular pain and weakness, rise of temperature, slowed pulse and increased susceptibility. (This author does not state how this last manifestation was determined, but one infers that the subject exhibited more severe symptoms on resumption of the habitual diet.) No albuminuria was found. The patient recovered in a few days after changing his diet.

Collins and Martland[5] report peripheral neuritis with eventual recovery as a result of repeated exposure to a solution of hydrocyanic acid of unknown concentration, used by a dishwasher for brightening silverware. Schlegel[6] found experimentally that repeated administration of sublethal doses of cyanides produced chronic cachexia without increased susceptibility.

Koritschöner[7] attempted to treat tuberculous patients with repeated exposures to hydrocyanic acid. Twenty men and ten women were exposed to varying concentrations of the gas every day for six weeks. Seven patients showed chronic intoxication, pharyngeal congestion, itching, salivation, frequent vomiting, progressive decrease in pulse rate, lassitude and drowsiness and finally albuminuria. These symptoms abated in from three to five days after discontinuance of the treatment, but returned upon its resumption.

Twenty patients showed some fever which gradually diminished as the treatment progressed, indicating some degree of tolerance. Pulse and respiratory

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rates decreased progressively. Hemoglobin and red cells remained normal. Twenty patients gained weight during the first three weeks, the others losing progressively, but rapidly regaining upon cessation of the treatment. The only symptom constantly noted during exposure was coughing.

This report of pharyngeal congestion constitutes the only instance found in the literature examined of anatomic changes in the respiratory tract resulting from the more common cyanides, though Winternitz[8] states that halogen cyanides produce pulmonary edema and congestion which is quite persistent, but this is evidently due to the halogen radicle.

More recently Fühner[9] has made a rather exhaustive study of chronic cyanide poisoning in connection with the use of hydrocyanic acid vapor as a germicide. He describes the symptoms of acute poisoning as consisting of salivation, metallic taste in the mouth, petechiae on the tongue, reddening of the forehead, general weakness, lowered blood pressure, thoracic muscular pains, general malaise, and vomiting. He further inclines to the view that repeated doses do not produce chronic poisoning but *may* increase susceptibility.

In consideration of this conflicting evidence a few experimental results are here presented. Seven dogs were exposed under constant conditions in an airtight chamber, to sublethal concentrations of hydrocyanic acid vapor, eight times during a pediod of ten days for periods varying from fifteen minutes to two hours.

During exposure, nausea, vomiting, defecation, and urination were constant manifestations, while occasionally there occurred prostration from respiratory paralysis, always with recovery. Respiratory rates were usually increased temporarily and in case of any approach to paralysis, heart rates were increased also but in all cases where the animals remained quiet during exposure there were no changes in either respiratory or pulse rates.

During this period the animals lost from 2 per cent to 11 per cent of total body weight. But most of this loss occurred from the third to the fifth days with no marked change thereafter, while two gained slightly during the remainder of the period. It is also of interest that the two dogs showing the greatest loss of weight did not show any marked symptoms at any time during the exposure except salivation. These facts indicate that, either the loss in weight was due to some unrecognized factor or that there was developed a certain degree of tolerance. Contrary to Fühner's results, there was no evidence of increased susceptibility.

The four dogs showing the greatest loss in weight also underwent progressive decrease in heart rate except a slight transient increase when examined after exposure. There was no marked change in heart rate among the other three. None showed marked cardiac irregularity. Autopsies revealed absolutely normal conditions of the lungs and respiratory tract.

It is regretted that the peculiar conditions under which the experiments were conducted precluded a more detailed study, but the results presented include the more marked gross manifestations. It was not possible to study the metabolism rates or to make any neurologic examinations.

CONCLUSIONS

These results check fairly well with the clinical reports cited, and from the sum total of evidence, it may be concluded that chronic symptoms occur only on repeated exposure, a severe exposure that is not fatal producing no chronic symptoms.

Long exposures to very low concentrations may produce symptoms of poisoning.

According to experimental results there were no manifestations of increased susceptibility but on the contrary some indication of a certain degree of tolerance.

There are no marked anatomic changes that can account for the symptoms; a longer period of treatment may produce changes in the nervous system.

Symptoms of chronic poisoning are those ordinarily associated with general cachexia.

Individual susceptibilities vary over a wide range.

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INDICANURIA AND ACETONURIA OF GASTROINTESTINAL ORIGIN*

By Eric R. Wilson, A.B., Los Angeles, Cal.

THE analyses of seventy-five specimens of urine of individuals in supposedly normal health on what they considered an average normal diet (the average intake of protein was on questioning found to be high) showed an indicanuria associated with an acetonuria in thirty-six out of the seventy-five specimens examined.

In all cases the urines examined were free from albumin and sugar.

Those specimens showing an indicanuria and acetonuria were all of higher specific gravity and deeper color than the normal urines. Five of the thirty-six cases showed a more or less persistent acetonuria even after the indicanuria had been cleared up by liberal catharsis and diet, extending over a period of from five to thirty-one days.

CASE	ACETONE	DI-ACETIC	INDICAN	ACETONE	DI-ACETIC	INDICAN	ACETONE	DI-ACETIC	INDICAN	ACETONE	DI-ACETIC	INDICAN	ACETONE	DI-ACETIC	INDICAN	ACETONE	DI-ACETIC	INDICAN	ACETONE	DI-ACETIC	INDICAN	ACETONE	DI-ACETIC	INDICAN	ACETONE	DI-ACETIC	INDICAN
	DAY		1			2			3			4			5			7			14			21			27			31			33
1	+	0	+	+	0	+	+	0	0	+	0	0	+	0	0	×	0	0	0	0	0										
2	+	0	+	+	0	0	+	0	0	×	0	0	0	0	0																
3	+	0	+	+	0	+	+	0	0	+	0	0	+	0	0	+	0	0	×	0	0	0	0	0							
4	+	+	+	+	+	+	+	+	0	+	+	0	+	+	0	+	0	0	+	0	0	+	0	0	+	0	0	×	0	0	0	0
5	+	+	+	+	+	+	+	0	0	+	0	0	+	0	0	+	0	0	+	0	0	×	0	0	0	0	0					

\+ Positive.
0 Negative.
× Suspicious.

As will be noticed from the above table, three out of the five cases which showed an acetonuria of longer duration than any of the other thirty-six cases cleared up nicely within fourteen days under the administration of alkalies and a somewhat restricted protein diet, while two of the cases seemed to resist treatment. In one case the acetonuria lasted up to the twenty-first day from the time when acetone was first noticed in the urine. The other and most persistent case of this series showed an acetonuria extending over a period of thirty-one days before it cleared up.

It was demonstrated that a prolonged acetonuria, as we believe of gastrointestinal origin, showed a decreased carbon dioxide combining power of the blood plasma. The Van Slyke apparatus and technic were used for this determination.

It was also noticed that in the last two above-mentioned cases the coagulation time of the blood was materially increased from one to three minutes above normal.

The following tests and technic were used.

Acetone: To 10 c.c. of urine in a test tube add about one gram of ammonium sulphate, 2 to 3 drops of a freshly prepared 5 per cent solution of sodium

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nitroprusside, and 2 c.c. of concentrated ammonium hydroxide which may be stratified or poured on the mixture. The presence of acetone is indicated by the slow development of a permanganate color. The delicacy of this reaction is 1 to 20,000.

Diacetic acid: Arnold's test. Two solutions are kept in stock. One is a 1 per cent aqueous solution of para-amido-acetophenon with 2 c.c. of strong hydrochloric acid in each 100 c.c. of the mixture; the other is a 1 per cent solution of potassium or sodium nitrite. Two parts of the first and one of the second solution are mixed together in a test tube and an equal bulk of urine added, and finally a drop of strong ammonium hydrate. A brown color usually appears in normal urines, but on the addition of several drops of strong hydrochloric acid the color changes to yellow while in a urine containing diacetic acid a purple color develops. Normal urines may show a red color as well as those containing very slight traces of diacetic acid, on shaking, however, the foam will show a violet color when small amounts of diacetic acid are present. This point is particularly emphasized by Waldvogel.

Indican: Obermayer's test. To 10 c.c. faintly acid urine in a test tube add an equal volume of Obermayer's reagent, and about 3 c.c. to 5 c.c. of chloroform. Place the thumb over the mouth of the tube and shake vigorously. On standing a few moments the chloroform will settle and it will assume a blue color, if indican is present. The intensity of the color will vary with the amount of indigo blue which has been brought into solution by the chloroform. Normally, the chloroform should assume only a faint blue color. In other words, normal urine contains a trace of indican.

Bacterial digestion of protein in the intestinal tract is of material pathologic significance, and the one in which we are particulrly interested is that of Tryptophane.

Ten c.c. of a solution of 1% mixture of amino acids with tryptophane predominating in quantity plus five grams of sodium chloride to 1000 c.c. of the mixture was inoculated with 3 loopfuls of a thirty-six hour pure culture of B. coli. Incubated at 37½° C. for 24 hours at the end of which time the mixture was filtered through two layers of filter paper and tested for diacetic acid by the Arnold test.

Results: Showed traces of diacetic acid. Showed positive for indole.

Under anaerobic conditions the intestinal bacteria have in general the power of splitting off the amino group, whereas under aerobic conditions they split off the carboxyl group. Under one, the other, or both of these conditions the protein molecule is broken down into more or less toxic substances; viz., indol and skatol, which are partly absorbed by the blood stream as such and their toxicity removed by their combination with sulphuric acid to form sulphates. The aromatic sulphates further combined with the potassium to form ethereal sulphates and as such are excreted in the urine. In the case of tryptophane by the combined action of bacteria it is probably converted into indol-proprionic acid, thence to indol-acetic acid and finally indol and skatol, this is then oxidized, converting these substances into indoxyl and skatoxyl. The indoxyl is excreted in the urine as potassium-indoxyl-sulphate. The indol which is not thus absorbed is excreted by the feces.

It is very probable, therefore, that at a point between the formation of indol-acetic acid and indol and skatol there occurs a process by which diacetic acid is

formed and absorbed as such by the blood. The addition of acids to the blood causes their immediate neutralization by one of the buffer salts of the blood, namely, the $NaHCO_3$ and the protein. The buffer substances in the corpuscles may also share in this neutralization of the acid. During this process, however, two important points are to be observed—the appearance of the acid ions and their rate of elimination. It is possible that where an acetonuria has existed or been in progress for any length of time the tissues may develop an abnormal affinity for the H ions, and such we believe, often to be the case.

This affinity would undoubtedly be increased in cases where, for some reason or other, the kidney function has been impaired and elimination delayed, and resist to a greater degree the average therapeutic measures employed in combating an acidosis.

We have lately observed an acetonuria existing with a pronounced indicanuria in colitis, ileo-colitis, and in both staphylococcic and streptococcic cellulitis.

To just what extent, however, an acetonuria of gastrointestinal origin might progress and the effect it would have on the organism as a whole if allowed to continue untreated for any length of time, we are not prepared to say, but we believe that this condition exists and is far commoner than is usually observed.

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ARSPHENAMINE AND NEOARSPHENAMINE

By Wm. A. Smith, Springfield, Ill.

ARSPHENAMINE and neoarsphenamine are two of the most important and most discussed therapeutic agents of the present day. They are striking examples of that newer type of therapy known as chemotherapy, to which we look for great possibilities in the future, in the treatment of infectious diseases. Since there are many phases to this subject, I shall not attempt a complete discussion, but shall limit myself to the main facts in current literature. It might be interesting first to consider briefly the origin and the present supply of these compounds.

ORIGIN

Before the discovery of the causative agent of syphilis, atoxyl was being used in the treatment of trypanosomiasis. Atoxyl is an organic arsenic compound (sodium salt of paraamidophenylarsenic acid) which was introduced by Thomas. When in 1905, Schaudinn discovered that a spirochete was the cause of syphilis, and since the spirochete is closely related to the trypanosomes, atoxyl was promptly taken up in the treatment of syphilis. This was followed in many cases by dangerous reactions in the body, especially blindness. Moreover Paul Ehrlich[1] showed that atoxyl was relatively inactive in the test tube, and he concluded that the atoxyl became a parasiticide in the tissues. This change he thought to be due to reduction of the arsenic from a pentavalent to a trivalent form. This lead him to experiment with allied arsenic compounds. In 1910[2] he succeeded in producing a compound, which was most toxic to the parasite, and least toxic to the human body—combining extreme parasitotropism with a low organotropism. He called this compound salvarsan, but it also became well known as "606," which was his diary number for arsenic compounds. Previous to 1914, all salvarsan came from the Farbwerke-Hoechst Co. of Frankfurt, Germany, where it was made under the supervision of Paul Ehrlich. This firm was protected both by product and process patents. Neosalvarsan, also known as "914" was introduced later; this is a derivative of salvarsan, and in some respects is a more efficient remedy. It also was made only by the above German firm. As a result, the products on the market were fairly uniform in their composition and properties.

PRESENT SUPPLY

During the war, shipments were stopped, and the supply in the United States was exhausted in 1915. Patent protection was withdrawn in the allied countries, and firms in England, France, Japan, and Canada began to manufacture these compounds. After the United States entered the war, the Adamson Bill was passed, granting citizens the right to operate patents owned by enemy aliens. The result was that several products were placed on the market, varying both in their chemical and their physiologic properties. It became necessary to exercise precaution in granting licenses to manufacture these products; for this reason the U. S. Public Health Service defined the properties of good preparations, and the

Federal Trade Commission formulated rules and toxicity tests which must be complied with, or the license of the manufacturer may be revoked. The firms[3] licensed at present are:

The Dermatological Research Laboratories, Philadelphia Polyclinic, whose products are called arsphenamine and neoarsphenamine.

The Takamine Laboratory Co., Inc., New York City, whose products are called arsaminol and neoarsaminol.

The Farbwerke-Hoechst Co., New York City, (at H. A. Metz Laboratories Inc.) whose products are called salvarsan and neosalvarsan.

The Diarsenol Co., Buffalo, N. Y., (Synthetic Drug Co., Toronto, Canada), whose products are known as diarsenol and neodiarsenol.

The product of the Dermatological Research Laboratory was known as Arsenobenzol, and was found to be identical with salvarsan in composition and equal to it therapeutically, by the council on Pharmacy and Chemistry of the A. M. A. Pollitzer[4] even claimed it to be superior to the imported product. This product was made under the direction of Dr. Schamberg, and was the first made in this country.

RULES FOR MANUFACTURE

On March 4, 1918,[3] the Federal Trade Commission adopted certain rules for the manufacture of these products; chief among these are the following:

1. These products are to be named and labeled arsphenamine and neoarsphenamine respectively. (Arsphenamine was also previously called arsphenolamine hydrochloride.)

2. The products are to be sold only in colorless glass ampules, containing an atmosphere of an inert gas.

3. The arsenic content of arsphenamine must be between 29.5 and 31.57 per cent.

4. The arsenic content of neoarsphenamine must be between 18 and 20 per cent.

5. Each lot must be tested for its arsenic content and toxicity.

6. For toxicity tests,[5] at least four or five albino rats are to be used—non-pregnant—weighing 100-150 grams. A 2 per cent solution of neutralized arsphenamine in doses not less than 80 mg. per kg. body weight is injected. It is required that 60 per cent of the animals injected survive at least forty-eight hours. Animals are not to be anesthetized for injection and if more than one rat dies, tests are to be repeated. Special rules are given for feeding and caring for the animals used. Tests are also made on samples submitted, by the U. S. Public Health Service. The Federal Trade Commission can withdraw from the market any lot designated. Detailed records of the tests for each lot are to be kept, and furnished to the Federal Trade Commission.

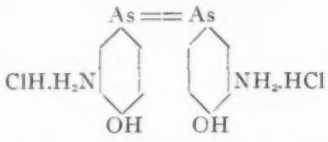
The maxium tolerated dose of neoarsphenamine must be not below 90 mg. per kg. body weight, with injection of a 2 per cent aqueous solution, intravenously, at the rate of 0.5 c.c. per minute.

Such restrictions have done a great deal to make the products on the market safe for use and have undoubtedly reduced the number of unfavorable reactions following injection.

CHEMISTRY

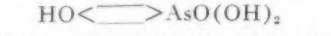
These products are derivatives of arsenobenzene.

Arsphenamine.[7] Its formula is $C_{12}H_{12}.O_2N_2As_2.2HCl$

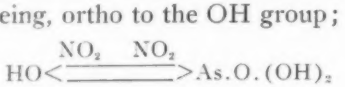


Arsphenamine; or dihydroxy diamino arsenobenzene hydrochloride; or para-diamido dioxy arseno-benzol hydrochloride; or 3 diamino 4 dihydroxyl -1 arseno-benzene hydrochloride.

It is prepared from parahydroxy phenylarsenic acid, which is:



(a) By addition of nitric and sulphuric acids obtaining a mono-nitro compound—the nitro group being, ortho to the OH group;

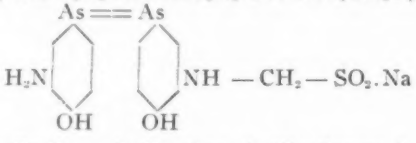


(b) then by reduction with caustic soda, sodium hydrosulphite and magnesium chloride producing arsphenamine. Two molecules of nitro compound produce one molecule of arsphenamine.

Among the reactions are the following:[7]

1. There is no effect with dilute mineral acids (except sulphuric)—unlike neoarsphenamine.
2. With dilute sulphuric acid a yellow precipitate is formed.
3. Concentrated mineral acids (except phosphoric) form a precipitate; in the case of nitric acid, the precipitate dissolves in excess, forming a red solution.
4. Carbon dioxide immediately precipitates arsphenamine from aqueous solutions.
5. Sodium hydroxide forms a precipitate, dissolving in excess.
6. Oxidizing agents as chlorine or bromine water or ferric chloride cause an aqueous solution to become red. (Same as neoarsphenamine.)
7. There are no inorganic arsenic compounds in pure arsphenamine, (or neoarsphenamine) which may be shown by obtaining no precipitate on addition of hydrogen sulphide gas to an aqueous solution. The molecular combination seems to prevent arsenic from combining with the tissues, preventing arsenical poisoning.

Neoarsphenamine.[7] Its formula is



Neoarsphenamine, or Sodium 3 diamino 4 dihydroxy-1-arsenobenzene methanal sulphoxylate.

It is prepared by addition of an aqueous solution of sodium methanal sulphoxylate to an aqueous solution of arsphenamine; a precipitate forms, which dissolves in sodium carbonate to form neoarsphenamine.

Among the reactions distinguishing it from arsphenamine, are these:[9]

1. Dilute and concentrated mineral acids cause precipitation.
2. Acetic acid, and heat, forms a yellow precipitate, unlike arsphenamine.
3. No precipitate forms with NaOH.

PHYSICAL PROPERTIES[8]

Arsphenamine	*Neoarsphenamine*
A Pale Yellow-microcrystalline hygroscopic powder.	An Orange-yellow, microcrystalline powder.
unstable in the air.	changes to brown in the air.
odorless.	odorless.
taste—sour.	taste—like garlic.
Soluble in H_2O, ethyl and methyl alcohol.	Soluble in H_2O, and glycerin (only slightly in alcohol).
The aqueous solution is greenish yellow and strongly acid to litmus.	The aqueous solution is neutral and yellow, turning brown on exposure to the air.

TECHNIC OF ADMINISTRATION

The preparation of arsphenamine solution is more complicated than neoarsphenamine, as it must be neutralized before injection. The drug is dissolved in cold water, freshly boiled and distilled (hot water is used in the "arsenobenzol" brand); this acid solution is then neutralized with 15 per cent NaOH[8]-0.9 c.c. for each 0.1 g. of drug and diluted so as to give a solution containing 0.1 ($\frac{1}{10}$) gram arsphenamine to 30 c.c. of final solution (McCoy[9]). Schamberg[10] states that dilution should be as much as 120 c.c. of water for each 0.6 gm. Guy[11] believes that dilution should be at least 0.1 gram to 15 c.c. of solution. Dilution with freshly distilled water is considered an essential point; to avoid untoward reactions. Injection of the acid solution is very painful and is also responsible for cases of toxicity, so neutralization is important. The solution should always be filtered through sterile cotton to avoid introducing solid particles into the blood.

Authorities agree that the time of injection should be relatively long. Guy[11] states that a dose that may be well borne by animals, when given slowly for 15 minutes, may be fatal when injected in one minute. McCoy[9] states that 2 minutes should be allowed for each 0.1 gm. (30c.c.) injected; thus for an injection of 30- 180 c.c., 2 to 12 minutes would be required. This makes it possible to stop the injection at any moment, if immediate reactions develop. At least on first injections, Bory[12] injects only 1 c.c., then waits 15 seconds and repeats.

Before using any specimen, the ampule should be immersed in 95 per cent alcohol for 15 minutes to detect apertures and cracks.[9] This is because the compound is unstable and easily oxidized by the air. If a breach is discovered, the contents of the ampule are to be discarded.

The gravity method of injection is used in preference to the piston syringe, with more safety to the patient. The drug is administered intramuscularly and intravenously. At first it was given intramuscularly by most physicians, and Leonard[13] still favors this method. The best site for this is at the buttocks. Most writers favor the intravenous method; it is less painful and more efficient. In treating syphilis of the nervous system it has been found helpful to withdraw at the same time a portion of the spinal fluid, so that the drug will reach the nervous system more quickly.

The technic of administering neoarsphenamine differs from that of arsphenamine in several respects. It is more soluble, and gives a neutral solution. Only one ampule should be dissolved at a time, after being tested for cracks, as neoarsphenamine is even more unstable than arsphenamine. Cold, freshly distilled water should be used. McCoy[9] believes 2 c.c. water to each 0.1 gm. of drug should be used. This compound may be injected in a piston syringe, using a very small needle. The injection may be more rapid, McCoy stating that it should be done in not less than 5 minutes, however.

Neoarsphenamine[10] should never be administered unless the solution is brilliantly clear; cloudy solutions produce immediate reactions with syncope a dominant symptom. This action seems to be mechanical.

REACTIONS AND THEIR CAUSE

Unfortunately, the use of these compounds has often been followed by serious reactions, and in a few instances by death. The causes of the reactions have been attributed to different factors in different cases. These are classified by Guy as due to drug toxicity, errors of technic, and a peculiarity of the patient. Some reactions were thought by Danysz to be due to a precipitation of arsphenamine base in the blood stream, owing to the carbonic acid present, since the amount of acid in the blood has a definite relation to the number and severity of reactions. Danysz[11] recommends small initial injections to vaccinate against reactions, by increasing the amount of organic bases in the blood. But this may produce a resistant strain of spirochete, blocking the treatment. It also means a loss of time. Herxheimer reactions have followed small injections, so Danysz's theory is not accepted (Guy). Some cases are due to oxidized products of arsphenamine, which are toxic. Thus ampules should be tested for leaks, and solutions should only be used soon after preparation. Chemically pure NaOH and freshly distilled water are essential.[3] Water containing much Ca and Mg causes more reactions than otherwise. Incomplete neutralization of arsphenamine solutions have accounted for more avoidable reactions than almost any other factor (Pollitzer).[4] The acid solution is 50 to 60 per cent more toxic than the alkaline. Filtration will prevent solid particles from getting into the blood.[5] Different brands of the drug vary in toxicity, and one should observe the directions of the manufacturer of that particular brand being used.[6] Minor reactions might be due to the fact that the lot *just* managed to pass the toxicity requirement. Some reactions are thought to be due to the destruction of enormous numbers of spirochetes, liberating the protein of their bodies in the blood stream and producing anaphylactic reactions. Guy[11] states that Herxheimer reactions are due to the stimulating activity of nonsterilizing doses, and are avoided by a full therapeutic dose. He believes that all other sources must be eliminated before attributing the cause to the drug. Vasomotor reactions occur often while injecting the drug, and these may be controlled by epinephrin, and the injection continued. Guy[11] reports three cases of thrombophlebitis due probably to infiltration of the drug between the coats of vessels. There was pain and tenderness along the course of the swollen and thrombosed vein, with a febrile reaction lasting for 10 to 20 days. The cases recovered, but the hardened and sclerotic vein persisted.

Auer[14] has shown that toxicity is inversely proportional to the acidity and concentration. Either because of the acidity of the injected arsphenamine or because of the increased protein content of the blood of certain syphilitics, an intravascular precipitate forms. This was shown by the fact that the serum of patients contained a substance, which precipitated with arsphenamine *in vitro;* this also probably occurs *in vivo*—causing the symptoms by subsequent vasodilatation, which is controllable by subcutaneous injections of epinephrin (Berman). Some of the symptoms appearing during injection are congestion and swelling of the face, or cyanosis of the face and congestion of the head, oppression of the chest, cough, dyspnea, and pain in the back. These may disappear when the injection is stopped. It has been stated[15] that arsphenamine and neoarsphenamine lead to reduction of the chromaffin tissue in the adrenals, hence to a less epinephrin content in the gland and in the blood—a selective action on the adrenals. Milian[16] objects to giving epinephrin in all cases, as this deprives the physician of a guide to the tolerance of the patient, and there is danger of giving too large a dose. Headache following injection may not be due to toxicity, but lacrimation, watery nasal discharge, and excessive secretion of saliva are signs of an impending "nitritoid crisis" or serous apoplexy. The temperature is a very reliable guide. If it rises above 100.4° F, Milian[16] states it is a sign of marked intolerance, and if it occurs after each dose, the drug should be discontinued. Jackson and Smith[17] recommend the use of tyramin in place of epinephrin as there is less danger of acute heart dilatation and the blood pressure does not rise so high. The effect with tyramin is also more lasting. Toxicity is not due to intermediate products in the manufacture of the drugs, as the Hygienic Laboratory of the U. S. Public Health Service tested these and found them nonpoisonous. These reactions may develop severely in suprarenal conditions (Addison's disease) or a thymolymphatic constitution. Hirano[18] states that the serum of animals injected with arsphenamine or neoarsphenamine contained less vasocontractile substance than normal serum; the content of both adrenals was decreased, the deficiency not being restored. Rieger states that toxic products may arise in the ampule.[19] He believes that arsphenamine insufficiently dried, in six months may develop toxic by-products in the ampule, the amount varying with the time before use. These specimens may pass the biologic test at the time manufactured, and later produce these toxic products. In one specimen, for example, the ampule was sound, but the contents gave a solution of a gun-metal tinge—due, to oxidation. (Arsphenamine will shed metallic arsenic when oxidized.) Arsphenamine, after being dried for two hours, contains about 7 per cent methyl alcohol. This alcohol may act on the base in the ampule and methyl arsin accumulate. This injected, will cause nitritoid reactions, even cardiac paralysis may result. Neoarsphenamine, made without the use of methyl alcohol does not have this danger. Some reactions have been clearly due, then, to the drug itself.

Of reactions due to technic, most of the causes have been mentioned. Many injections were made too rapidly, too large doses were given, and solutions were incompletely neutralized. Nelkin[20] objects to such high dilution of the drug. He uses 0.6 gm. to 20 c.c. of water and reports no untoward effects. He states that a slow injection is required when a large quantity is given, but otherwise not. He also uses the syringe method, which is more simple and time saving. Guy thinks that injection of a solution too cold accounts for some reactions. Others are due

to improper preparation of the patient. He reports 80 cases in all stages of infection, injected without preparation and all developed gastrointestinal reactions. This preparation should include: urinalysis—for evidence of kidney irritation, (one case of death, showed an acute diffuse hemorrhagic nephritis.) A cathartic should be given the evening before, very little food given, and the patient should remain quiet after the injection. All reactions can not be avoided by fixed rules of technic, as no case falls in any well-defined group. Thus different workers have had success with different methods.

Even with the best technic and a comparatively nontoxic drug, reactions will occur, which have their origin in the patient. Guy cites 40 cases in which reactions were due to aggravation of nonsyphilitic pathologic conditions, as bronchitis, bronchopneumonia, and icterus. He also cites two cases due to idiosyncrasy, in which the symptoms were gastrointestinal with signs of prostration. In some cases, the reaction was delayed; injection was followed by no effects up to the fourth day. There was greater prostration in these cases. Reactions are not due to sensitization,[14] since attempts to experimentally sensitize guinea pigs have failed; also because the effects are produced with *one* injection. Incompetent renal, hepatic or pulmonary functions account for many reactions, where the relative functional integrity of organs is upset. If the temperature of a patient is elevated he will not take the drug well. The status of the infection is thought also to influence the reactions. Among the reactions reported, are these:

Neurorecurrence, by Jacobsen,[21] and Berner.[22]

Arsenical eclampsia, by Bory.[12]

Toxic dermatitis, by Nelkin.[20]

Hemorrhagic purpura, by Labbè and Langlois.[23]

(They state that bleeding of gums and nose, and patches of purpura should warn one to stop the treatment.)

Gangrene of the fingers, by Stutter.[24]

Jaundice, by Nagai.[25]

Exfoliative dermatitis with gangrene of toes, by Jones[26]—due to arsenical deposition in the system, causing obliterative arteritis.

Milan[27] thinks that arsphenamine has no affinity for the liver, and that jaundice is due to the action of syphilis; it can best be conquered by pushing the treatment. Sicard[28] disagrees with him, and states that arsphenamine does damage the liver, reflected in the urea content of the blood.

Myocarditis, extensive nephritis (nonsyphilitic) and diabetes are contraindications to arsphenamine.

USE IN THERAPEUTICS

These drugs, while used primarily in syphilis, have been found of value in several nonsyphilitic diseases. Prominent among these are frambesia, relapsing fever and Vincent's angina, all being due to a spirillum. This has led Best[29] to conclude that arsphenamine is specific in diseases caused by any spirillum. Its aid in treatment of amebic dysentery, anthrax and malaria, has led him also to conclude that arsphenamine has curative properties in other diseases where the organism is easily reached, as in the blood and lymph. Brechot[30] reports using neoarsphenamine intravenously, after war wounds, in order to prevent and to

cure septicemia, especially acute septicemias without important local lesions. The results were very successful. De Beaurepaire[31] used neoarsphenamine with excellent results in yellow fever. He gave an initial dose, as large as 0.9 g. Head and Seabloom[32] call attention to the fact that syphilis may prolong and delay reparative processes, such as in the lungs of persons having had pneumonia. They cite a case, where resolution was delayed for months. Under arsphenamine treatment, recovery was rapid.

Schamberg[10] states that the best treatment for syphilis is organic arsenicals and mercury. Writers differ in the strength of the dose, the frequency of administration and choice of compound. The trend today, is to make the treatment at first as intensive as is consistent with safety, for the best chance of recovery. The curability of syphilis is directly proportional to the age of the disease.

The first injection kills the mass of parasites, but a few survive and may reinfect the tissues. Mercury is used to avoid tolerance to the drug. Mercury acts slower and longer; thus by combining the two, one gets quick action as well as prolonged action. This is shown by the fact that most of the arsphenamine is excreted in three days, while a sterilizing concentration of mercury is reached only after several days.[1]

Sicard[28] gives neoarsphenamine intravenously in a daily dose of 0.15 g., to a total of 6 to 7 g. in women, and 7 to 9 g. in men. He thus gives 40 to 60 injections in all.

Tauber[33] injects 0.3 to 0.6 gm. every 3 to 7 days, for 4 to 6 injections. Combining this with mercury, he gives no less than three such courses in two months. He points out that congenital syphilis requires longer treatment.

Nelkin[20] gives an average dose of 0.5 gm. to the male and 0.4 gm. to the female. He repeats this weekly for three doses, then two more doses at longer intervals. He gives mercury and iodine between these injections.

In animals[1] a fall of blood pressure follows injections; this has been met with in case of man. Large doses in the rabbit seem to induce nephritis. The after effects in man are usually headache, nausea, vomiting, chills, and often fever. Delbanco[34] used silver arsphenamine in 550 injections with excellent results. Silver has a special affinity for the spirochete, shown by the silver stain. It banished the spirochetes from the primary lesion, as shown by the dark field microscope, and the florid symptoms of the secondary period subsided promptly. He also used mercury with the silver arsphenamine. The advantage of this compound is that the maximum effectual dose is 0.2 to 0.3 g., which is below the threshold of danger, as the arsenic content of silver arsphenamine is only two-thirds that of arsphenamine. He concludes by saying that silver acts chemically as a catalyzer of the arsenobenzol molecule, and biologically as a reenforcer of the specific action of arsenic on spirochetes. Colloidal silver alone has a pronounced action on rabbit syphilis.

The elimination of these compounds has also been observed. Myers[35] states that one-half hour after injecting arsphenamine, 75 per cent of it is fixed in the body cells. Arsenic is found attached to proteins of the plasma and blood cells, even after all free arsphenamine is eliminated.

Arsphenamine[1] appears unchanged in the urine in 5 to 10 minutes after injection, and persists for 5 to 6 hours. Thereafter arsenic is found in urine for

several days, as arsenites and arsenates. Less is eliminated in the stools, but it appears there for a longer time. No arsenic is found in any organs after 15 days; 50 to 75 per cent of the arsenic may be regained in the urine. The fate of the remaining 25 per cent is unknown, but appears to be temporarily stored in the liver and bone-marrow. With intravenous injection, the arsenic disappears in 3 to 4 days; with intramuscular injection it disappears from the body in 10 days.

Neoarsphenamine elimination differs from that of arsphenamine in that for the first few hours after injection, formaldehyde appears in the urine. Otherwise it is eliminated in the same manner as arsphenamine.

RELATIVE VALUE OF ARSPHENAMINE AND NEOARSPHENAMINE

I will conclude with a brief discussion of the merits and demerits of each compound. For a long time arsphenamine was used dominantly, but during the war, all treatment at the front, by French, English, American and German doctors was with injections of neoarsphenamine. This must have given better results.

Toxicity—Schamberg[10] showed that neoarsphenamine is two and one-half times *less* toxic for white rats than the arsphenamine from which it is made. Arsphenamine was tolerated at 100 mg. per kg. body weight, while the neoarsphenamine was tolerated at 250 to 300 mg. per kg. body weight. The addition of the formaldehyde sulphoxylate group apparently lessens the affinity for the protoplasm of the parasite, but also lessens the affinity for the body proteins still more. This is due to the closing of one amino group (Schamberg).

Further, arsphenamine in practically all concentrations hemolyzes red blood cells in vitro. Neoarsphenamine does not do so, in any concentration normally used (Schamberg).

The hydrogen-ion content of neoarsphenamine is close to that of the blood; that of arsphenamine, whether alkaline or acid, differs greatly from that of blood. Neoarsphenamine is usually better tolerated than arsphenamine. Milian[16] states that neoarsphenamine never induces nitritoid crises except in extremely predisposed and intolerant persons.

Curative Powers.—Arsphenamine is more curative than the neo-compound. Schamberg[10] found that to sterilize rats experimentally infected with Trypanosome equiperdum (horse syphilis) it took twice as much of the neo-drug as arsphenamine itself. The arsenic content of three parts of neoarsphenamine, equals that of only two parts of arsphenamine.

Schamberg[10] thus concludes, that the arsphenamine is more active therapeutically, but this difference is made up by the discrepancy in tolerated dosage. Nine-tenths gm. of the neo-compound can be given three times a week. Neoarsphenamine creates much less biochemical disturbances in the blood and tissues.

The value of these drugs[1] has been shown by the destruction of spirochetes in the test tube, and also by the fact that this power is greatly enhanced when digested with tissues—inorganic arsenic compounds are presumably formed. We might ask, if these drugs will actually cure syphilis. Authorities differ, but Schamberg[36] has presented an interesting case in support of the curability of this disease. He believes patients treated in the primitive stage intensively may

be cured. In this case syphilis was acquired in 1917. The Wassermann was positive. After treatment by neoarsphenamine, the blood gave a negative Wassermann, and the eruption disappeared. In 1919, syphilis was again contracted, a chancre developed at a site different from the first one. The blood now gave a strong positive Wassermann. Schamberg believes the facts warrant the assumption that the first infection had been cured. No immunity was given against the second attack, however. Too much value should not be given to the Wassermann reaction, in deciding the question of curability.

At any rate, under such treatment, if applied early, external lesions disappear; secondary signs, as alopecia, and secondary syphilitic systemic disturbances are prevented.

Tauber[33] crystallizes the view of writers, when he states that "mercury and arsphenamine must be combined in the rational cure of syphilis."

Now that the supply is carefully controlled, and as a result of the investigations as to the cause of reactions, it is hoped that these valuable compounds will be used with better success by the average physician. It might be desirable to further modify them to form a new compound specific for the spirochete pallida. The success of these compounds, on the whole, has given new hope for the cure of all infectious diseases,—by the elaboration of chemical compounds, which will combine a high parasitotropism and a low organotropism in each case.

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PRIMARY CANCER OF THE LIVER

By I. M. Cohn, Spokane, Washington

PRIMARY malignancy of the liver is a very rare condition. In fact almost every known case has appeared in some medical journal. Many of these cases have been challenged so that the number of instances of this disease is very small.

Maude Slye observed but four cases in 1500 autopsies. While experimenting with mice she made 10,000 autopsies. Twenty-eight showed primary tumors of the liver. Of these only 3 were malignant, one of which produced multiple metastasis in the lung.[1] On the average, it occurs in about 0.1 per cent of autopsies. It is rarer than secondary malignancy. The secondary condition occurs with 20 to 40 times the frequency.[2]

Wheeler reports 15 cases in 5,233 autopsies which were performed at Guy's Hospital in London.[3] Vecchi reviewed 45 cases of so-called primary carcinoma of the liver. Of these he considers only 21 as being correct. The others were doubtful.[4]

The etiology of this disease is unknown. Many theories have been advanced any or all of which may be the direct or indirect cause. The frequency with which the disease is found with cirrhosis of the liver has led many to believe it to be the factor causing the disease. Dickenson reports a case in which the liver was removed from a man with alcoholic habits. It was a mass of cancerous tissue combined with an older coarse cirrhosis.[5] Frequently it has the hobnailed appearance characteristic of cirrhosis.[6] One of the rarest conditions was cited by Hale White. It was the development of a melanotic sarcoma in the liver following cirrhosis. Only three or four cases of primary melanotic sarcoma of the liver have yet been obtained.[7] W. Ford reports a case of a spindle cell sarcoma found in the cirrhotic liver of an alcoholic.[8] In the five cases of malignant adenocarcinoma examined by Muir, all were associated with cirrhosis.[9] Where malignancy of the liver follows cirrhosis, there is no doubt but that the cirrhosis has been a great factor in bringing out the malignancy.[10] All cases of cirrhosis can be considered as potentially malignant, and that cancer is very often the sequela. Some take the view that cirrhosis is the sequela of cancer.

An interesting case is cited by M. DeMassary. His patient was a young French soldier of 25. On autopsy an enlarged liver was revealed. It was syphilitic. The inflammatory reaction set up resulted in cirrhosis, and this in turn gave rise to a malignant carcinoma which caused his death.[11]

Other irritating conditions are important factors in causing the disease. G. Rohdenburg found a round cell sarcoma during an autopsy, the primary nodule being formed about a dead parasite in the liver. There was no doubt but that the parasite was the primary foci.[12] Another interesting case was that of an autopsy on a Chinaman. His liver was covered with carcinomatous masses. On microscopic examination, leprosy bacilli were observed scattered through the

stroma of the liver. Previously it was not believed that two such diseases could exist together in the same liver.[13]

Biliary calculi have been associated with carcinoma. There are two views on their relationship. The first, or irritative, theory considers the calculi as the cause of carcinoma. The other, or concentration, theory assumes that the growth causes the calculi. Primary carcinoma arises most frequently in the region of the gall bladder. In most instances gall stones are present, while in secondary carcinoma they are seldom found. The fact that gall bladder cells may show carcinomatous growth in the liver supports the irritation theory. Beadles cites thirteen cases of malignancy, all of which were associated with gallstones. However, these cases may have been confused with primary carcinoma of the gall bladder.[14] A rather far-fetched view is advanced by Fischer. He considers primary cancer of the liver as originating in the biliary passages, as cirrhosis of the liver is present in 85 per cent of the cases of adenocarcinoma of the biliary passages.[15]

Arteriosclerosis has been advanced as a cause. Of the four cases examined by Warner, only one showed evidence of arteriosclerosis. In no instances have vascular changes been proved to be the cause of cancer. Cirrhosis has been the condition most frequently associated with malignancy.[16]

There are three important types of carcinoma of the liver, massive, diffuse, and nodular.[17] The massive type occurs most frequently. It consists of a large carcinomatous mass inside the liver so that the liver tissue acts as a shell for the neoplasm. The tumor cells are usually polyhedral or spheroid. The diffuse type of carcinoma is very rare. In this condition there is an infiltration of spheroidal cells into the liver tissue. The nodular, or multiple primary, carcinoma is more common. It grows rapidly, and quickly degenerates. In this condition the liver has the appearance of being studded with nodes. The cells are polyhedral or spheroidal. Primary carcinoma in a cirrhotic liver is a fourth type, while primary melanotic carcinoma is a fifth type.

Primary sarcomas are divided into five classes similar to carcinoma. Primary massive sarcoma is usually found in the right lobe or pedunculated. The tumor is made up of small round cells, spindle cells and other types. The nodular or multiple sarcoma is made up of spindle, round, irregular or giant cells. In diffuse sarcoma the tumor cells infiltrate both lobes of the liver. Sarcoma also arises in association with cirrhosis. More rarely we have primary melanotic sarcoma of the liver.[18]

A description of a typical sarcomatous liver is described by Delepine.[19] An enlarged liver was removed at autopsy. The surface was nodulated like that of a hobnailed liver. These nodules varied in appearance. Some were white while others were dark brown. A microscopic examination of the tissue revealed considerable changes in the intralobular veins. There was a proliferation of endothelium to the extent of occluding the vessels. Some of these vessels contained golden brown pigment granules and others contained black pigment globules. Ninety per cent of the liver epithelium was destroyed, while many of the remain-

ing cells were pigmented. The larger pigmented nodules were made up of spindle cells.

The tumor is set in action by an irritation of the liver. This leads to a proliferation of endothelial cells in carcinoma, and of connective tissue in sarcoma. The contents of the vessels are replaced by an embryonic tissue similar to that from which blood and blood vessels originate. Along with this is the rapid destruction of normal liver tissue.[19] An examination of the carcinomatous cells show them to be quite similar to hepatic parenchyma. They have a trabecular arrangement, but the cells are larger than normal tissue.[20] The tumor is a direct continuation of normal tissue though it contains many large and irregular cells.[21] In many tumors the cells are bile-producing, as is found in normal tissue.[22] The tumor can be considered as a direct change of liver cells to cancer cells, and a hyperplasia of the new formation.[23] The growth is compensatory and it proliferates by direct extension. It is this factor which gives rise to malignancy.[24]

Metastasis with cancer of the liver comes at a later period than with malignant diseases elsewhere in the body. Neither are the metastases so widespread. The retroperitoneal glands and omentum are frequently involved, but seldom the supraclavicular glands.[25] The lungs are often involved and very rarely the heart.[26]

Primary cancer has a wide variation. It occurs at almost any age in an individual's life. Several cases have been reported in which the disease was present at birth. Pepper reports the case of a baby girl who died with a congenital lymphosarcoma at the age of six and one-half weeks.[27] In this case there was a metastasis to the right suprarenal body. The symptoms were mistaken for congenital syphilis, and only at autopsy was it proved to be a congenital round-cell sarcoma of the liver.[28] Similar cases have been reported in infants by Heaton[29] and Pitt.[30] It may remain latent and not cause death for several years.[31] As a rule, though, it runs a rapid course, death usually occurring within a month after the onset of the symptoms.[32] In these cases of congenital cancer the individual inherits the predisposition to the disease.[33] It occurs more often in the form of a carcinoma than a sarcoma.[34]

In children the disease has been mistaken for intussusception and appendiceal abscesses so rarely does it occur.[35] In most cases the disease is first noticed by an abdominal swelling. A hard lump is felt under the right rib margin. The tumor continues to grow and extends below the rib margin. The enlargement of the liver pushes the other organs to the left. This has often caused it to be mistaken for a cyst and punctures have been unsuccessfully made to relieve the condition.[36] It was only at autopsy that the true character was made out.

The symptoms vary greatly in different cases. Clinically the earliest symptoms are vague gastrointestinal troubles. Sometimes the symptoms of an atrophic cirrhosis predominate. After the tumor develops the patient suffers a loss of flesh, cachexia and digestive disturbances. Icterus is present in 63 per cent of cases; ascites in 58.5 per cent; edema in 41 per cent; splenic tumors in 32 per cent cases and fever in 14 per cent.[37] Eighty-six per cent of the cases

are associated with cirrhosis. It is also associated with echinococcus cysts, chronic venous congestion, and tuberculosis of the liver. On the whole it occurs most often when the liver tissue is undergoing regeneration.[38] Emaciation, vomiting, and constipation are symptoms frequently present. The tumor area is usually painful and tender.[39]

The duration of the disease seldom exceeds twelve weeks. If longer, it is more likely to be secondary. The prognosis of the disease is fatal, death almost always results. It is somewhat more common in the male. The family history of the patient is usually negative. Although the liver is usually greatly enlarged it may be smaller in cirrhosis.

A typical case as found in children is cited by Martha Wallstein.[41] A four-month-old baby was brought to the clinic with a history of having vomited for the past three days. On examination, a small lump was noticed under the right lower rib. Further examination produced negative results. During the following two months the child became worse, the abdomen was markedly distended and death resulted at the age of 8½ months. The autopsy revealed a primary malignant sarcoma of the liver. In one case the abdominal swelling continued over a period of eight months.[42] Castle reports a case in which death took place within fourteen days after the onset of the symptoms.[43] An examination of the tumor showed it to be a primary parenchymatous adeno-carcinoma of the liver. Tate reports a similar case in a ten year old girl.[44] Cases are given by Bramwell,[45] Affleck,[46] Roberts.[47] Two cases are reported of boys, one who gave a history of scarlet fever and nephritis,[48] and the other[49] of being kicked in the right side. The symptoms were characteristic. The body was covered with eruptions, the patient became weak and emaciated. Diarrhea and abdominal swelling set in, and the patient died in great pain. On autopsy primary malignant growths were found in the liver.

The cases reported are found at all ages and in both sexes.[50-62] The disease has also been found in mice quite similar to the human form.[63] Very rarely misplaced tissue in the liver may become malignant. In these instances the tumor resembles the tissue from which it is derived. A primary myeloma in the liver was found which had myelogenous functions.[64]

Although this is a very malignant disease, operations have been resorted to with some success.[65] This has been done by cutting the tumor away and scraping out the cavity.[66] In more radical operations the entire affected lobe of the liver has been successfully removed.[67, 68] Where recurrence occurred a second operation was resorted to with more success.[69]

SUMMARY

Occurrence: Very rare.

Etiology:

1. Irritation of liver cells.
2. Congenital origin.
3. Compensatory hyperplasia.

Kind:[3]

1. Liver cells.
2. Bile duct cells.

Symptoms:

1. Cachexia.
2. Anemia.
3. Digestive disturbances.
4. Right hypochondriac pain.
5. Tumor in abdomen.
6. Icterus when bile exits are occluded. 63%.
7. Ascites when lymphatic obstruction occurs. 58%.
8. Edema. 41%.
9. Splenic tumor. 32%.
10. Fever. 14%.

Duration: Fourteen days to a year. Averages six months.

Age: Occurs at any age.

Sex: More common in men.

Macroscopic classification:

1. Nodular—most common.
2. Massive.
3. Diffuse—rare.

Microscopic classification:

1. Simplex.
2. Adenomata.

Metastasis:

1. Liver.
2. Lungs.

Weight: May weigh as much as 267 ounces.

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THE EFFECT OF TOBACCO ON THE VASCULAR WALL

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THE inhalation of tobacco or injection of nicotine causes a contraction of the artery. This can be shown by the rise of blood pressure. From observations on eight healthy men Lee[1] concludes as follows: In the novice there is always an initial rise of systolic blood pressure. In an excessive smoker the pressure rises and the pulse is unaffected.

The rise of blood pressure may be due either to a change in the heart rate or to the increase in output or to an indirect effect associated with the respiration. Miller, Bruce, and Hooker,[2] from observations on two healthy men, conclude that after smoking (pipe, cigar or cigarette) there is usually an increase in heart rate. Aikman[3] made an experiment on 28 healthy smokers, using a single Turkish cigarette in each case. Of 27 cases, the pulse-rate increased in all but 4, and 16 showed an increase of more than eight beats per minute. Stachelin[4] attempted to ascertain the effect of smoking continued over a period of six months. During this time he smoked six to eight cigars daily, then he abstained from smoking for six months. The pulse rate, as showing by the mean rate on three days was 82 during the smoking period and 75 during the period of abstinence. Similarly the pulse rate after a standard exertion was 157 compared with 124 per minute. Not only was the pulse rate after exertion higher during the smoking period, but it took much longer to return to the normal. This showed that the heart during smoking period was more irritable and its output might be affected. Hesse[5] observed the effect of cigar smoking on 25 subjects, chiefly patients with various chronic diseases. One reading was taken before smoking and one after each cigar. In the majority there occurred an increase in pulse rate, together with a rise of blood pressure. Although experiments have shown that tobacco may affect the heart rate or its output, there are also experiments which appear to prove that the rise of blood pressure may be caused by a vasoconstriction resulting from the inhalation of tobacco.

Aikman[3] observed the effect of cigarette smoking on the pulse rate and blood pressure of 28 subjects, chiefly healthy smokers. In four heavy smokers the pulse rate increased by 40 beats. He noted an initial decrease of 4 to 6 beats in three cases. The rise of blood pressure and the change of pulse rate did not run in the same direction.

Bruce, Miller, and Hooker,[2] studied carefully two healthy smokers and concluded that after smoking there was generally an increase in heart rate. Even when the increase was absent, the blood pressure showed distinct changes. The maximum and minimum presure always rose slightly, the maximum the more, so that the "pulse pressure" tended to increase. This is the case when the rise of blood pressure is caused by a vasoconstriction, therefore the increase in pressure may be independent of the heart rate. Moreover, Lee,[1] studying

healthy men, stated that in the case of an excessive smoker the pressure rose and the pulse was unaffected.

John Parkinson[7] made a serious attempt to ascertain the effect of smoking. There was a sudden increase in the average rate during the first half minute of smoking from 80 to 87. No preliminary slowing took place, such as has been described in the case of animals after the introduction of nicotine. In five cases the blood pressure did not rise until ten or more minutes had elapsed. The average diastolic pressure rose gradually. Among the inhalers the average systolic pressure rose 11 mm.; the diastolic pressure rose 6 mm.; the systolic pressure rising more than the diastolic. This shows that the vasoconstriction played an important role in some of his experiments.

The effect of injection of nicotine upon the vascular wall can also be shown by the rise of blood presure. Some writers have thought that the effect of nicotine upon pressure might be indirectly due to the increase in the output of epinephrine. Gley[8] gives a figure showing a rise of pressure from 30 mm. to 140 mm. mercury, following injection of 10 mg. of nicotine into a nine kg. dog with intact adrenals, whereas after the excision of the adrenals, the same dose of nicotine only caused a rise of 24 to about 30 mm. He concludes that the whole pressor effect of nicotine after destruction of the bulbospinal center is due to the increase in the output of epinephrin produced by it. Dale and Laidlaw[9] observed that certain reactions which are elicited by nicotine on the non-pregnant uterus of the cat and in the eye after removal of the superior cervical ganglion are modified when the experiment is made under such conditions that epinephrin can no longer reach these structures from the adrenals. They explain the difference by the hypothesis that the nicotine action is in part due to a stimulation of the adrenals to increase liberation of epinephrin. Cannon, Aub and Binger[10] conclude that "injection of nicotine of small amount (3.5-7.5 mg. in cats) results in augmented adrenal secretion." But such an increase of concentration of epinephrin in adrenal veins is always observed when the blood flow in the cava is slowed from any cause whatever, provided that the epinephrin output continues unchanged or is diminished less than the rate of the blood flow. It is possible that the increase of concentration is owing to the diminution of the rate of blood flow associated with the marked and prolonged fall of blood pressure succeeding the very brief rise.

Langley[11] obtained an excellent rise of pressure when nicotine was injected after the adrenals had been tied off from the circulation. Here, of course, the bulbospinal centers may have participated in the action. It may be assumed that if nicotine stimulates the ganglion cells on the path of the adrenal secretory fibers, or in the absence of ganglion cells on the path of these fibers[12] possibly some part of the medullary cells themselves, an increased epinephrin output may contribute to the rise of pressure. But all the evidence goes to show that even if the output has been proved to be augmented by nicotine and the adrenal blood after being collected for a time is suddenly released, the epinephrin factor in the increase of pressure is far subordinate to the vasomotor factor. It would appear improbable that the excitation of ganglion cells on the cause of vaso-

motors should be a negligible factor in the vasoconstriction in comparison with the excitation of the adrenal secretory fibers.

Lauder Bronton[13] has given the following statement: In animals nicotine causes slowing of the heart with enormous rise of blood pressure. The rise of pressure is chiefly due to the contraction of arterioles. This contraction is partly dependent upon the stimulation of the vasomotor center in the medulla oblongata partly also to a local action upon the arterioles themselves, as it is produced by the injection of the drug after the medulla has been destroyed.

As it has been stated that nicotine causes an augmented adrenal secretion, it must have some action on veins. For, as an accepted explanation, epinephrin acts on muscles which are supplied by sympathetic autonomic nerve fibers, and veins receive such kind of nerve fibers. The portal vein receives vasoconstrictor fibers from the splanchnic nerve.[14] The existence of venomotor nerves to the large veins of the neck has been proved.[15] Bancroft[16] made experiments in which it was found that stimulation of the sciatic nerve caused a constriction of the superficial veins of the hind limbs. Moreover it has been described that nicotine does have an action on the vasomotor center.[13] It is not impossible, therefore, that nicotine may act on veins through its own action on the vasomotor center as well as through the action of epinephrin.

The effect of tobacco upon vasoconstriction may be shaded somewhat by the fact that tobacco attacks the hemoglobin. Claude Bernard and Hobert Amory Hare have shown the effect of nicotine, pyridine and gross tobacco itself on the blood. The action consists of attacking the hemoglobin in the red corpuscles of the blood. This fact has been confirmed by Clark, who reports that the hemoglobin content may fall as low as 40 per cent. The largest portion of the oxygen is held in chemical combination with the hemoglobin.[17] In other words, the amount of oxygen in the arterial blood depends upon the amount of hemoglobin there is in one cubic centimeter of blood.[18] When the hemoglobin content falls the oxygen supply is insufficient and certain organic acids, such as lactic acid, may accumulate. Gaskell has shown that acids in slight concentration cause a vascular dilatation. Bayliss[19] has stated that lactic acid or carbon dioxide may act to produce a dilatation. So the vasoconstriction caused by tobacco may be shadowed somehow by its indirect action on the vasodilatation.

CONCLUSION

The inhalation of tobacco causes a contraction of the artery.

The rise of blood pressure after smoking may sometimes be due to a change in heart rate, sometimes due to the vasoconstriction independently.

The injection of nicotine causes a vasoconstriction.

Such vasoconstriction is due either to the augmented adrenal secretion caused by nicotine, or to a direct, local action of nicotine on the vascular wall, or to its action on vasomotor center.

Tobacco affects not only the artery but also the vein.

The effect of tobacco on the vascular wall may be shaded somewhat by its action on hemoglobin and consequently a vasodilatation may come to neutralize its result to some extent.

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LABORATORY METHODS

THE SELECTION OF KIESELGUHR FOR THE FILTRATION OF SERUMS*

By George E. Ewe, Philadelphia, Pa.

IN THE filtration of expensive serums, every possible means of preventing loss must be carefully investigated. One of the avenues of loss is absorption by the filtering medium used to clarify serums.

One of the most commonly employed filtering mediums is kieselguhr. Kieselguhr varies greatly in its power of absorbing liquids. Trials of seven lots of ignited kieselguhr, under precisely the same conditions, showed the following results of relative power to absorb serums:

KIESELGUHR	LOSS OF SERUM
1	2.28%
2	5.7%
3	5.9%
4	2.43%
5	4.3%
6	4.8%
7	2.39%

Therefore, the relative values of these samples of kieselguhr, for the filtration of serum, regarding minimum of loss by absorption, were in the following order: No. 1, No. 7, No. 4, No. 5, No. 6, No. 2, and No. 3.

It is also essential to select kieselguhr which will yield a brilliantly clear filtrate in a minimum of time. The seven kieselguhr samples mentioned above showed great variation in this respect. Table I gives the results of filtration experiments with various lots of serums under exactly the same conditions.

Since the time required for final filtration through a Berkefeld is too short for accurate comparison, only the figures obtained by preliminary filtration through filter paper can be used for comparing the relative value of these seven lots of kieselguhr regarding their ability to produce clear filtrates in a minimum of time. Analyzing the results outlined in the above tables, we find the relative values to be as shown in Table II.

Table II shows the varying ability of different lots of kieselguhr to clarify the same lot of serum. It also shows a fair uniformity in the ability of a particular kieselguhr to clarify different lots of a serum.

Chemical examination of the seven samples in accordance with the tests for kieselguhr in the United States Pharmacopeia yielded the results shown in Table III.

*From the Pharmaceutical Chemical Laboratories of the H. K. Mulford Co., Philadelphia, Pa.

TABLE I

KIESELGUHR SAMPLE NO.	SERUM NO.	MINUTES REQUIRED FOR PRELIMINARY FILTRATION THROUGH PAPER	MINUTES REQUIRED FOR FINAL FILTRATION THROUGH BERKEFELD
1	1	20	3½
	2	21	2
	3	23	1½
	4	20	2
	5	19	2
2	1	55	6
	2	60	5
	3	60	6
	4	120	5
	5	78	5
3	1	35	4
	2	60	2½
	3	120	3½
	4	60	15
	5	66	5
4	1	53	2
	2	47	10
	3	50	5
	4	60	9
	5	50	2
5	1	60	4
	2	18	2
	3	25	7
	4	25	4
	5	25	16
6	1	45	2
	2	37	10
	3	30	5
	4	45	2
	5	50	2
7	1	12	1
	2	15	1

TABLE II

	SERUMS				
	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5
NO. OF KIESELGUHR SAMPLE	7	7	–	–	–
	1	5	1	1	1
	3	1	5	5	5
	6	6	6	6	4
	4	4	4	3	6
	2	2	2	4	3
	5	3	3	2	2

These chemical results do not present evidence of sufficient difference in the chemical nature of the seven samples of kieselguhr to account for the variation shown in the value of the samples for the filtration of serum.

Therefore, chemical examination alone seems to present no means of selecting kieselguhr for the filtration of serums.

Microscopic examination did not present a method of comparison for valuation of relative ability of kieselguhr samples for serum filtration.

TABLE III

KIESELGUHR	COLOR	ORGANIC MATTER	LOSS ON IGNITION
No. 1	Yellowish-White	Trace present	11.4%
No. 2	Grayish-White	" "	10.3%
No. 3	" "	" "	10.4%
No. 4	" "	" "	10.4%
No. 5	" "	" "	5.2%
No. 6	" "	" "	10.3%
No. 7	" "	" "	10.9%

So we are reduced to the necessity of actually comparative filtration experiments regarding loss of serum by absorption, rate of filtration, and clarity of filtrate. In addition, chemical examination is necessary in order to insure absence of excessive organic matter and moisture.

Credit is due the Serum Filtration Department of the Mulford-Biological Laboratories at Glenolden, Pa., for conducting the filtration experiments mentioned in this note.

A CONVENIENT STOPCOCK-NEEDLE-CANNULA*

BY PAUL J. HANZLIK, M.D., CLEVELAND, OHIO

THE small instrument illustrated in the accompanying figures represents a combination of hypodermic needle and small metal stopcock intended for use as a cannula. The cannula is made by soldering a needle to one end of a simple, one-way, nickel-plated, brass stopcock, with a short tube at the opposite end for admission of the tip of a Luer syringe. The weight of the cannula is about 6 grams.

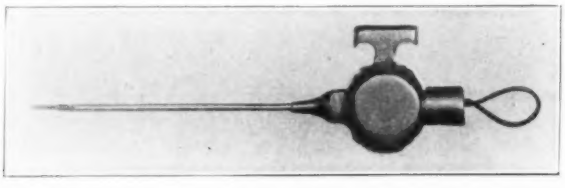

Fig. 1.—Photograph of large size stopcock-needle-cannula; actual size.

I have found the stopcock-needle-cannula very convenient in connection with research and demonstrations for class work, particularly when repeated injections are necessary. The needle portion of the cannula is inserted directly into an exposed vein in the usual way and then secured by a ligature or serrefine clip. For larger veins (saphenous, femoral, jugular, etc.) of larger animals (cats, dogs, etc.) the larger cannula of Fig. 1 is more convenient. For the ear veins of rabbits and guinea pigs the smaller needle-cannula of Fig. 3 is more desirable. This can be securely held in place by means of a small serrefine clip, which encloses the edge of the ear with needle in place.

*From the Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, Ohio.

The technic of injection by means of the stopcock-needle-cannula is best carried out as follows: Before insertion into the blood vessel, the instrument is filled with normal salt solution (0.9 per cent NaCl) by means of a Luer syringe. This is easily done by turning the stopcock to the transverse position while saline is being forced through. This is done to exclude air and avoid embolus formation. The needle is then placed into position by direct insertion into the vessel and secured by means of a ligature or serrefine clip as described above. The tip of the syringe, which now contains the solution to be injected, is inserted into the cannula and the stopcock quickly opened, and the contents forced in, closing the stopcock (transverse position) when the syringe is emptied. When very small volumes (fraction of a c.c.) are injected, about 1 c.c. of saline should be injected at once in order to wash out the remnants in the needle.

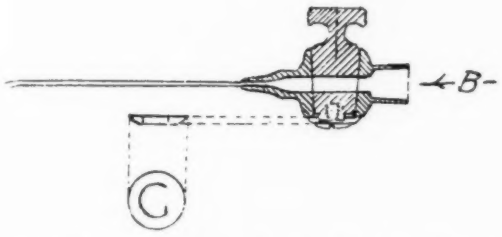

Fig. 2.—Sectional view of large size stopcock-needle-cannula; actual size; *B*, for insertion of Luer syringe.

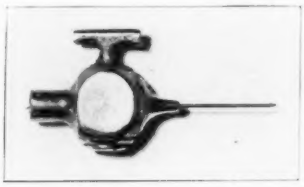

Fig. 3.—Photograph of small size stopcock-needle-cannula; actual size; fits Record syringe.

The stopcock-needle-cannula possesses the following advantages: It replaces the more cumbersome glass cannula and burette, with connections. It is nonbreakable. It is serviceable for a wide range of different sized blood vessels, avoiding the maintenance of a large assortment of glass cannulas. It is convenient for making quantitative injections of small volumes of fluid, and small doses of substances, thereby avoiding complications from the injection of additional wash fluid necessary with burettes or other larger apparatus. For instance, in studying the effects of agents on cardiac volume, such a needle-cannula is obviously very convenient, if not indispensable. Larger volumes of fluid can also be injected, since Luer syringes of different sizes fit the instrument equally well.

There is no difficulty with clot-formation in the needle when solutions are injected into the circulation. I have not tried the needle cannula for repeated withdrawal of blood to see whether clot formation would hamper this. However, by insertion of the stiletto this should be easily remedied.

In case the needle should break off, a new one can be easily soldered into the stopcock by a technician of ordinary ability.

The stopcock-needle-cannulas are obtainable from the Geo. H. Wahmann Mfg. Co., 520 West Baltimore St., Baltimore, Md.

I am under obligations to our technician, Mr. M. Dan, for the actual construction of the cannulas here presented, and to Professor T. Wingate Todd for the photographs.

A NEW URINOMETER*

By Jacob Rosenbloom, M.D., Ph.D., Pittsburgh, Pa.

At the last meeting of the American Medical Association, there was demonstrated a new urinometer that I devised. The accompanying illustration shows this instrument.† It requires no separate jar, no pouring of the urine back and forth, and can be used when there are only small amounts of urine available.

In using the urinometer, one draws the specimen from container by compression of the bulb and the specific gravity is then read off from the hydrometer as in the usual method.

*From the Laboratory of Dr. Jacob Rosenbloom, Pittsburgh, Pa.
†Made by the Scientific Materials Co., Pittsburgh.

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EDITORIALS

The Present Status of Our Knowledge Concerning the Etiology of Influenza

THE medical profession met the great present pandemic of influenza which began in 1915, and has extended to the present day, with no definite information as to the possibilities of a specific method of prevention and therapy. There had been, to be sure, a gradually increasing and an almost settled conviction that the microorganism that Pfeiffer described near the latter end of the last great pandemic of this disease (1892) was really the causative agent involved. It will be recalled that the B. influenzæ was found by Pfeiffer in certain, but by no means all, groups of cases that were to be classed under this malady of many aspects and complications. In subsequent sporadic outbreaks Pfeiffer's bacillus has been found or not as the case might be, success depending in no small measure on the bacteriologic skill involved. This factor of technical difficulty is still of importance in the more recent and otherwise skillful bacteriologic work of the last months. It is, however, by no means sufficient to explain the shifts of opinion and consequent confusion that have arisen concerning the cause of influenza.

Fatal respiratory complications were recognized in the studies of previous influenza epidemics, but it seems certain that they were never so manifest as in the successive waves of the disease that swept the world from 1915 to the present

time. The varied bacteriologic causes of the pneumonias that have terminated so many recent cases of influenza have confused the issue as to the primary etiologic agent of the disease itself. It is now apparent that we must separate the primary cause of that prostrating but relatively nonfatal disease known as influenza from its pneumonic sequel of which one or several bacteria may be the cause. It seems now certainly proved that influenzal pneumonias may be produced by several very different microorganisms, among which the most important are the several types of Pneumococci, the Streptococci, both hemolytic and nonhemolytic, the influenza bacillus of Pfeiffer itself, Staphylococci and the bacillus of Friedländer.

The primary etiology of influenza is by no means certain. It is not in the province of this note to attempt to sift in detail the evidence on which the fluctuating scientific opinion in regard to influenza etiology has been based, but to anticipate our argument it may be stated that in the beginning of the present pandemic Pfeiffer's bacillus was generally accepted as the primary cause of influenza, that its etiologic rôle later became rather generally discredited and that finally it would seem in recent months to have regained to a considerable extent its former importance. The data in which these shifts in viewpoint have been founded may now in their salient features concern us.

In discussing the etiology of influenza, attention should again be called to the importance of distinguishing between the microorganisms found in the upper respiratory passages and those detected in the organs, particularly in the pneumonic lungs, of fatal cases. It should further be stated that the difficulty of determining what constitutes a primary pneumonia and what a postinfluenzal pneumonia, still further complicates the question. The organisms found in the upper respiratory passages we may assume represent most nearly the primary causative agent, and those found in the lungs may mean merely secondary invaders.

As a result of a survey of the literature on the bacteriology of influenza the following tentative conclusions may at present be drawn: In the first place, the bacillus of influenza has been found by a large number of observers in the upper respiratory tract of cases of influenza. Among these observers may be mentioned Wolbach,[1] Opie, et al,[2] Medalia,[3] Hickox and Gray,[4] Duval and Harris,[5] Pritchett and Stillman.[6] The percentage of positive results obtained from cultures by these observers varies, but may in many instances approximate 100 per cent. It should be stated that the influenza bacillus has also been found in normal cases by certain observers, notably Opie, et al,[7] in as high as 35.1 per cent normal individuals.

The influenza bacillus is by no means so frequently found in the pneumonic lungs of fatal influenza cases, though by some observers and in some epidemics it has been found there as the predominating organism (Opie, et al,[2]) and (Duval and Harris[8]), and it may unquestionably produce a fatal pneumonia. The blood cultures are almost always negative in such cases (Lamb[9]).

Various other organisms, such as the various types of the pneumococcus, streptococci, Friedländer's bacillus and the like, may be the cause of postinfluenzal pneumonia, and they may also be found in predominating numbers

in the early cases in the upper respiratory passages. These findings, however, by no means indicate that any one of them is the cause of the primary disease.

The possibility arises and has been directly asserted by a number, for example by Nicolle and LeBailly,[10, 11] by Gibson and his coworkers,[12, 13] da Cunha, et al,[14] and by Yamanouchi and others[15] that neither the influenza bacillus nor any other organism found in the throat or lungs is the veritable, primary cause of influenza, which in their opinion is a filtrable virus.

As bearing on the etiology of influenza the most important evidence of artificial reproduction of the disease is still in dispute. Many observers have completely failed to produce anything resembling influenza, either in man or in animals by inoculating cultures of influenza bacilli, other bacteria, filtrates from cultures, and also respiratory secretions from influenza cases (Wahl, White, and Lyall,[16] Friedberger,[17] Rosenau,[18] and others).

On the other hand, toxic substances formed by the influenza bacillus have been demonstrated by Parker,[28] by Huntoon and Hannum,[29] and by Albert and Kelman,[30] and lesions in some respects suggesting influenzal processes, have been produced with these toxins by the latter two authors. It has recently been claimed by Blake and Cecil[19] that a pneumonia resembling in all respects influenzal pneumonia can be produced in monkeys by the inoculation of pure cultures, and Davis[20] claims to have produced influenza in man by massive doses of influenza bacillus.

Further evidence bearing on the etiologic role of B. influenzæ is given in numerous reports of the presence of the serum reactions in influenza patients with this microorganism. Agglutinins have been described in influenza cases by Wilson,[21] Fleming,[22] Spooner, Scott, and Heath,[23] Gay and Harris,[24] and Duval and Harris,[25] and fixation antibodies have been described by Rapport[26] and Kolmer, Trist, and Yagle.[27] The question arises as to whether these reactions are etiologically specific or reactions of some nonspecific type.

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—*F. P. G.*

Infections and Vaccines

IT IS evident to the observer that vaccines are a very popular form of treatment of infections. It is also as evident that in not infrequent cases the principles of vaccine treatment are hazy in the mind of the physician. The situation is one which causes wonder whether the bacterial substances are being used as a last resort or whether they are being used principally on the chance that they may do good and so save time for the busy practitioner. It seems, therefore, advisable to outline briefly and as practically as possible the principles of vaccine therapy.

In general we may designate a microorganism infectious if it is able to multiply and produce symptoms of disease in the animal body. In order to produce disease it must enter the body, and in so doing it must overcome obstacles, some mechanical, others functional, of the cells and fluids of the host. The rapidity and extent of the invasion depends, in part, on the readiness with which the organism assumes a parasitic existence in the host, the site of entrance into the body, the size of the initial dose, and the resistance of the invader to the attack

of the defensive forces of the host. Particular races and strains of an organism may require special qualities by which they are able better to gain entrance to and maintain themselves in a special organ of the host,—"organ affinity;" or during their residence in the host they may be so modified that they are no longer susceptible to the previous lethal action of body fluids,—"serum-fastness;" or they may come to occupy positions of the body relatively inaccessible to the defensive substances of the body. The recognition of these latter factors is of great importance in devising and applying therapeutic measures.

Vaccination has for its aims: (1) The initial increase of resistance of the host so that invasion by bacteria is made more difficult or, in the extreme case (small pox), impossible. (2) To so modify the fluids of the body that once an organism has invaded the body it is not able to survive the condition which discourages parasitism (typhoid). (3) To so modify the fluids that in the case of developed parasitism, the bacteria are eventually killed (furunculosis). It is this latter instance in which we are especially interested at this time.

In considering cases of established infection (parasitism), there are three circumstances to regard: (1) localized infection, (2) general infection, and (3) infection not strictly localized.

In the first case (localized infection) it may be assumed that the reason the infection is localized is that the local (tissue or organ) conditions are such that the growth of the parasites is inhibited to a certain extent because of a locally acquired protective mechanism. That the mechanism is not a general one is shown in certain cases, for instance furunculosis, in which when one or a group of organisms is set free from the original focus a new focus is established in which, as in the first, a local partial immunity or resistance is developed. If, in such a condition, the injection of a substance into the body can cause a sort of mobilization of immunity resources or an increase in the general resistance to the organism concerned in the infection, the local mechanism of defense will be enhanced and the bacteria killed, and at the same time wandering organisms will not find satisfactory conditions for growth and multiplication and will be removed by the destructive cells of the tissues. If we consider furunculosis as a chronic infection associated with a series of acute infections, then what the vaccination does is to immunize prophylactically against succeeding new infections while at the same time assisting in the cure of the old.

In the second case (general infections, especially in streptococcic and staphylococcic sepsis) the use of vaccines has not been brilliant, possibly because the body is already overtaxed with substances of the same character in those injected. The logical conclusion in such cases would be that vaccination is apt to have harmful effects rather than beneficial ones.

In the third case (infections not strictly localized) one is dealing with a group of borderline cases for which it is impossible to formulate any satisfactory rules. In general, Irons says, "the more closely the case approaches a strictly localized infection the more likely it is to prove amenable to vaccines." It is in this group of cases that the possibilities of doing harm by ill advised inoculations are most strikingly evident from a clinical point of view. The delicate balance of aggressive and defensive forces is easily disturbed and an infectious process hitherto confined to one or more foci may be converted quickly

into a general infection, temporary perhaps, but accompanied by multiple new metastases.

Theobald Smith commenting upon the general problems of vaccination, says "all parasites tend to increase the resistance of the host in which they live and multiply. Out of this universal fact a number of practical problems arise. In any given disease is it worth while to try to raise this immunity and how much energy will it cost the patient? If worth while, what is the best and most sparing way of raising such immunity artificially? In any localized infection you must ask: Is this a beginning process without attendant immunity, or is it a residual process associated with general immunity? If the latter, then vaccines may be considered safe. In processes associated with fever and bacteriemia science says 'Hands off' until we know whether we have a progressive disease with gradual undermining of the resistance, or a more localized affection in which the excursions into the blood are secondary. Judged from this point of view as well as from the work of the laboratory, we would say that vaccines applied during disease will be rarely, if ever, life saving, but that they may hurry a stationary or languid process which tends toward recovery by bringing into play the unused reserves of the various tissues."

To summarize: Infectious processes in general are suitable for treatment by active immunization, (1) if they are localized, i.e., confined to one or more isolated lesions and are not associated with bacteriemia; (2) if they are more or less chronic.

Vaccines are suspensions of killed bacteria in salt solution, oil, or water, with usually a small amount of preservative, such as phenol or tricresol added.

Autogenous vaccines are prepared from bacteria isolated from the patient who is eventually to receive the vaccine. To quote Irons again. "Assuming that we have a case which clinically is suitable for treatment by active immunization, we may inquire what preparation of bacteria will be most efficient in stimulating the inactive forces of immunity. The researches of recent years indicate that one of the chief elements in the chronicity of an infectious process is a change in the infecting organism by which it becomes less susceptible to the attack of the forces of immunity of the host, and that this bacterial resistance or 'fastness' may be as important as the deficiency in the formation of antibodies of the host. Another similar change in the infecting organism, the acquirement of 'organ specificity' by which through long sojourn in a particular organ of an animal, or series of animals, the invader develops a special aggressiveness for that organ must also be reckoned with. If artificial active immunization is to be effective, it should aim to take cognizance of all, or of as many of these elements as possible, and hence a vaccine should be derived from an organism possessing the properties of those concerned in the infectious process,—an autogenous vaccine."

Stock vaccines are prepared from strains of bacteria isolated at some previous time, and kept in the laboratory in stock. They have no direct biologic relationship with the clinical case in which they are used. The foregoing definition taken with the discussion of autogenous vaccines indicates the reasons for the probable disadvantages of stock vaccines. Nevertheless, there are certain cases in which the stock vaccines are productive of good effects. Wherever this is true, it is also probably true that an autogenous vaccine would be more valuable.

Mixed vaccines are vaccines made of all the microorganisms present in a secretion or in a material. Their value is questionable and their use unscientific. One writer says, the resort to mixed vaccines, particularly to mixed stock vaccines, is either a confession of ignorance of the exact nature of the infection and misapprehension of the principles of immunity or an evidence of indifference and laziness on the part of the physician using them. It were more advisable to use a known unmixed stock vaccine, a carefully studied polyvalent vaccine, or a single purified protein.

Finally and as practically as possible, vaccines (autogenous) seem to be useful in the following infectious disorders:

1. Furunculosis and localized abscesses in soft tissues.
2. Acne vulgaris.
3. Colon bacillus pyelitis and cystitis.
4. Chronic gonorrhea and gonorrheal rheumatism.
5. Chronic bronchitis.
6. Bronchial asthma of bacterial origin.

Vaccines seem to be of little or no value in,—

1. Infection of bone or infections in cavities with rigid walls.
2. Infection of the intestinal tract.
3. Infection of the uterus and adnexa.

Vaccines are contraindicated in,—

1. Acute infections and infectious diseases.
2. Septicemia and pyemia in the acute stages.
3. Malignant endocarditis.

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—*P. G. W.*

The Therapeutic Value of Oxygen

THERE is no therapeutic measure that is less efficiently put into practice than the administration of oxygen, and as a consequence, most physicians have little faith in its value. There are several reasons for this state of affairs: in the first place, the physiologic mechanism by which added oxygen could assist in the respiratory functions is not understood; in the second, an insufficient amount of the gas is usually given, and in the third, it is usually given too late.

On the other hand, when oxygen is properly administered in suitable cases before the patient has become moribund, much evidence has accumulated to show that very great benefit indeed results from the treatment, and so far as can be told, a fatal termination is often averted.[1, 2, 3]

In order to understand the physiologic principles involved in this treatment, it is important to remember that although forty to fifty times as much oxygen is combined with hemoglobin as is in simple solution in the blood plasma, yet it is the latter which really diffuses into the tissues. The pressure of oxygen in the plasma, in other words, the diffusion pressure of oxygen, is the determining factor in causing it to permeate the tissues, and whenever this pressure begins to

fall under normal conditions, more is added to the plasma by dissociation from the oxyhemoglobin of the corpuscles. The plasma retails the oxygen to the tissues, and the corpuscles are the wholesale warehouses from which the plasma replenishes its stock. The unloading of oxygen from the oxyhemoglobin to the plasma is assisted by various chemical changes that take place while the blood is in the capillaries.

From these considerations it follows that an efficient supply of oxygen to the tissues could be maintained without any hemoglobin if we were to put an excess of the gas into simple solution in the plasma; that is, if enough were forced into solution in the plasma in the lungs so that the tissue requirements could be met without any local addition from oxyhemoglobin. Two experiments may be quoted to show that it is possible to do this.

1. After replacing all the blood from the blood vessels of a frog by artificial plasma (Ringer's solution) the animal can be kept alive for days in a vessel containing pure oxygen (i.e., five times the amount present in air) and during this time the rates of O_2 consumption and CO_2 production are practically the same as normally.

2. Animals (mice) exposed to air containing more than 0.5 per cent of carbon monoxide (contained in coal gas) soon become moribund, because the oxygen carrying power of the hemoglobin is abolished by the formation of carboxy hemoglobin. If the animals are now transferred to pure oxygen under two atmospheres pressure (i.e., ten times the amount in air) they quickly recover, and after leaving them under these conditions for some time, one may then transfer them from the oxygen to air without the toxic symptoms returning. If, on the other hand, they are transferred from the oxygen to air immediately after recovery, the symptoms of asphyxia return and the animal dies.

Both experiments show clearly that if we succeed in getting sufficient oxygen into simple solution in the plasma, the oxyhemoglobin is not necessary to supply the tissues with this gas.

It is evident, therefore, that oxygen administration can be of no avail in poisoning by coal gas or any other substance which destroys the O_2-carrying power of hemoglobin, unless it is forced into the alveoli so as greatly to increase the partial pressure.*

In pulmonary edema, in "gassed" cases, in bronchitis, and in decompensated cardiac cases, oxygen is also of undoubted value, when it is properly administered. Many cases of pneumonia are also benefited by such treatment, but there are others in which the heart is so profoundly affected by toxic substances that oxygen may perhaps be of little use. Evidence of this benefit is afforded by the easier and deeper breathing, the slowing of the pulse, the disappearance of cyanosis and the greater ease and comfort experienced by the patient. Not only do these effects persist as long as the gas is given, and thereby serve to tide over a crisis and permit the natural defensive agencies of the body more successfully to combat the abnormal condition, but they often outlast the administration.

Quantitative evidence that in pneumonia the arterial blood is improperly saturated with oxygen in proportion to the degree of cyanosis, and therefore of the condition of the patient, has been furnished by Stadie.[4] In a normal person

*Hemoglobin becomes converted into methemoglobin and incapable of carrying labile O_2 in various types of poisoning, e.g., acetanilide, nitrobenzol.

the arterial blood carries 95 per cent of its full load of oxygen (i.e., it is 5 per cent unsaturated), but in pneumonia it may carry only little over 80 per cent. Meakins[5] has confirmed these findings, and has added most important observations on the effect of oxygen inhalations by the Haldane method. In a case of pneumonia the oxygen unsaturation on the eighth day of the disease was 17.9 per cent. After two hours of oxygen treatment (at a rate of delivery of 2.5 liters O_2 per minute) the unsaturation percentage fell to 9.08, and after eighteen hours (at 1 liter per minute) it was 9.0. The O_2 was then discontinued and in four hours the unsaturation percentage had risen to 15.5. It fell subsequently to 3.05 after twenty-four hours, during which 3 liters O_2 per minute was administered. Shortly after this the crisis occurred. Similar results were obtained in a case of chronic bronchitis, and even in normal men it was found that administration of 3 liters of O_2 per minute for 100 minutes changed the arterial blood from 4.4 per cent unsaturation to 1.87 per cent.

To explain exactly how the oxygen acts in this group of cases several possibilities must be considered. In the first place we must suppose that the respiratory membrane has become greatly reduced in extent because the alveoli in certain parts of the lungs have become more or less filled with fluid or exudate, although not entirely consolidated. Under these circumstances the blood during the time that it is circulating in the vessels of the affected portion of lung can not be reached by a sufficient amount of oxygen to saturate its hemoglobin fully, because there is an inadequate diffusion pressure of oxygen to penetrate the thick layer of fluid between the alveolar air and blood. So long as the hemoglobin is not fully saturated, the tension of gas in the plasma must become very low, and little can be available for the tissues when the blood arrives at them. When excess of oxygen is breathed, the amount which goes into solution in the fluid will become proportionately raised, so that there will be a much better chance for a sufficient amount to reach the blood so as to saturate the hemoglobin and create a proper tension in the plasma.*

The blood which leaves the lungs as a whole is a mixture of the more or less still venous blood from the affected portions and of arterial blood from the healthy portions, and it may be considered that the mixture is just on the borderline of being adequate to supply the oxygen requirements of the tissues and nerve centers—otherwise the animal could not live. A very little improvement in the oxygen supply will therefore suffice to turn the tide, and it is possible that this may reach it by diffusion through the fluid that has collected in the alveoli. By increasing the pressure of oxygen in the inspired air, more will become dissolved in the fluid (by Henry's law) so that the pressure gradient from air to blood through the fluid will become steeper. But another factor must be considered, namely, that the increased partial pressure in the healthy alveoli has caused more O_2 to go into simple solution in the plasma of the blood circulating in these portions, and although this can not cause the hemoglobin to carry away any greater a load of the gas, yet when this blood is mixed with that from the pathologic lobes, the dissolved oxygen will assist in bringing the hemoglobin up to its proper degree of saturation with oxygen.

*The coefficient of solubility of oxygen in water at 20° C. is 0.34 (i.e. 0.34 c.c. O_2 will diffuse through 1μ (.001 mm.) of water in 1 minute when 1 sq. cm. of the water is exposed to 1 atmosphere of the gas). (Krogh, A.: Jour. of Physiol., 1919. lii. 391.) The amount which will diffuse through fluid is proportional to the thickness of the layer of fluid.

It has been imagined by some that it is useless to give oxygen because the dissociation curve of the blood at varying pressures of the gas shows that even after reducing the partial pressure of oxygen to one half that obtaining in normal alveolar air, the blood still takes up about 80 per cent of its full load. It is argued that it is therefore futile to attempt to increase the oxygen carried by the blood by raising the partial pressure in the air which is inspired into the still healthy alveoli. From what has been said above, however, it is clear that this viewpoint does not take into account two important effects which follow when the partial pressure of the oxygen is increased, namely, that it diffuses much more rapidly through the fluid in the pathologic portions, and that more goes into simple solution in the plasma circulating in the healthy portions.

When the exudation completely fills the alveoli or a portion of the lung completely collapses, the blood ceases to circulate through the capillaries of the affected part, so that all the blood is passing through the capillaries of healthy parts, and the arterial blood may remain of a bright red color. In such conditions it is unlikely that administration of oxygen can be of any value.

The success of treatment with oxygen in suitable cases must depend on several factors, the most important of which are: (1) To get as much of the gas into the alveoli as possible. (2) To start the treatment early before irreparable damage has been done because of anoxemia. (3) To maintain the administration until cyanosis disappears. With regard to the first of these factors, it has sometimes been thought that there is an element of danger in giving too much oxygen. This depends on the observations made by several investigators that animals that have been caused to live in atmospheres containing an excess (80 per cent) of oxygen for some time develop symptoms of pulmonary irritation, leading on to pneumonia.

At percentages just short of this, however, namely, between 60 and 70 Karsner[6] found in rabbits that there was no evidence of pulmonary inflammation even after 11 days continuous exposure. This makes it highly improbable that there could be any danger in man even when the administration of oxygen was pushed to extremes.

The importance of early administration is evident when we realize that the damage of oxygen deficiency on the nerve centers and the tissues usually develops insidiously, and that once started the damage must lead to a progressive deterioration of the vital functions of the body. The respiratory center is among the first to suffer from the anoxemia. The result is shallow and rapid breathing. Such breathing does not, however, properly ventilate the alveoli, so that the anoxemia becomes aggravated, and a vicious respiratory circle becomes established. The defensive agencies of the body against toxins and bacteria are also depressed by the anoxemia so that resistance to the further progress of the disease is deteriorated. It is also possible that prolonged oxygen deficiency, or it may be some toxic substances appearing in the blood as a result, renders the hemoglobin less capable of carrying oxygen by changing some of it into methemoglobin. It is at least significant that this compound is formed in animals after massive injections of streptococci.[7] The maintenance of an adequate tension of oxygen in the plasma by administration of oxygen by the lungs must greatly assist in guarding against these effects.

For similar reasons, the administration should be maintained until all signs of deficient oxidation are removed, the best index of this being the color of the face. So long as there is any anoxemia, this is of a characteristic pale ashen hue, different from that of ordinary capillary congestion.

Regarding *methods of administration,* it may be said at once that the common clinical practice of placing a funnel connected with an oxygen tank in front of the patient's face is worse than useless. At the rate at which the oxygen is usually applied by this method, it is inconceivable how any measurable increase in the percentage of oxygen in the alveolar air could be attained, and if enough gas is turned on really to have some influence, the waste due to diffusion into the air is prohibitive.

Where no special apparatus is obtainable for the administration, the best method is to pass a wide gum elastic catheter into one nostril, through which the gas, after bubbling through water in a flask, is passed as quickly as is comfortable for the patient. The method is rendered much more efficient if the open nostril is closed by the attendant during each inspiratory act. Dr. Rudolf and I have found by this latter method that the percentage of oxygen in the alveolar air can be raised to 35 per cent when the opposite nostril is compressed during inspiration. With the opposite nostril open, this percentage was only slightly raised above the normal.

When special appliances are available, a choice may be made between the mask and valve devised for the purpose by J. S. Haldane,[1] and which was extensively used in the treatment of gassed men, or the hollow tongue depressor of Meltzer.[2] In the Haldane method the oxygen is discharged from a cylinder of the gas, (provided with a reducing valve) through tubing connected with a wide bore T-piece. To one limb of the T a small rubber bag is attached, and the other is furnished with a mica valve and ends in the face mask. The valve does not open on expiration, so that oxygen collects in the bag, and is inhaled on inspiration. The appliance is simple and saves oxygen, but patients not infrequently object to covering up of the face with the mask.

A very satisfactory method is that of S. J. Meltzer, in which a flat metal tube (hollow tongue depressor) is connected by wide rubber tubing to a very easily manipulated respiratory valve, beyond which is a strong rubber bag attached to the rubber tubing coming from an oxygen cylinder. When the valve is in the inspiratory position, the gas passes through the bag into the tongue depressor, when in the expiratory position it fills the bag and none gets beyond the valve. The tongue depressor is inserted in the mouth not much farther than the middle of the tongue, so that there may be no gagging or other discomfort, and the lips are kept closed. The valve is manipulated by the attendant about 10 to 12 times a minute. By this method, with the nose clamped, we have been able to raise the percentage of oxygen in the alveolar to eighty-five.

Of course by far the most satisfactory method is to place the patient in a respiratory cabinet filled with oxygen. Such cabinets are being tried in England, and there is no doubt that they will soon come into frequent use.

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—*J. J. R. M.*

The Chemistry and Physiology of Memory

LIPKIN has collected the literature on this subject from which we make the following abstract. The sum total of mental processes which alone is representative of the values that differentiate human existence from that of its humbler associates might be found, upon analysis, to be composed of a group of memory-ideas, through whose coordination and cooperation thought is made effective. No thought is possible which does not derive its material from memory-ideas. No memory-idea is possible which has not at some previous time entered consciousness as a corresponding sensation. Sometime, somewhere, an impulse, that had its origin in an effective stimulus in the external world, entered the brain by way of the sensory paths, produced a sensation, and, though its effects may have long since disappeared from consciousness, left a record which has never been completely eradicated and upon which our thoughts—through our memories—become conditioned. Pillsbury says: "Memory, imagination, reasoning, are limited in the qualities that they make use of to the bare materials of sense. They may recombine them, they may make use of the sense materials in new ways, but they can add no new qualities." Hence, memory resolves itself into the conscious experience of awareness of a group of centrally aroused sensations, sensations already existing in the central nervous system, retained as a result of changes produced in the nerve tissue by some previous stimulation. Explanation of the nature of this retention of memories and their reproduction as well as an analysis of the various factors that go to make up the structures of memory, will be attempted in the following:

Let us assume that we are remembering two simple sensations, one visual—yellow; the other, auditory—middle C. Let us follow these two sensations from their origin to their destination and attempt to arrive at the causes that make recollection of them possible. Waves of light are of different lengths, being composed of vibrations that range from 400 to 800 millimicrons in length. All light waves, the length of whose vibrations does not correspond to that of yellow, which is 554 millimicrons, are absorbed; all others are reflected to enter the eye. These vibrations affect the retina at the rate of 542 trillion times per second. They hit the rods and cones, and thus excite in them a visual impulse. This impulse travels through the bipolar cells, ganglion cells, and along the fibrils of the optic nerve with a velocity of 123 per second. We follow the impulse along the optic tract, and, after partial crossing at the optic chiasm to the external geniculate bodies, where connection is made with a second series of neurons that carry the impulse to the nerve cells of the visual center, which is

situated around the calcarine fissure of the median surface of the occipital lobe. Here the impulse excites the nerve cells into activity, resulting in visual sensation.

Sound is composed of vibrations emanating from vibrating medium, as, for example, the vocal cords that are set into vibration when the air passes up the respiratory passage. When middle C is sounded, 132 vibrations reach our ear every second, and set into vibration the tympanic membrane, the hammer, anvil, stirrup, the lymph within the cochlea, and finally the hair-like process of the basilar membrane—18,000 to 24,000 in number, some of which respond in sympathetic vibration with the vibrations of middle C. The sympathetic vibration thus excites the nerve cells of the spiral ganglia into activity and sets an auditory impulse traveling along the auditory nerve. We follow the impulse along the fibrils of the auditory nerve to its connection with a second layer of neurons, either in the ventral root of the eighth nerve or in the tuberculum acusticum, thence to the superior olive lemniscus lateralis, internal geniculate bodies, and finally to the nerve cells of the auditory center in the superior temporal gyrus and the transverse gyri, extending from this into the fissure of Sylvius. Here the impulse excites the nerve cells into activity and the result is an auditory sensation.

In these two illustrations we have two sensations—one produced by the excitation of the nerve cells of the occipital lobe, the other by the excitation of the nerve cells of the temporal lobe. In both cases the sensations were due to the impulses that have reached the brain by way of the visual and auditory paths. Experiments have shown that both impulses are essentially alike in quality; that if the auditory nerve be attached to the eye and the optic nerve to the ear, lightning would be heard and thunder seen, that the particular sensation experienced does not depend upon the stimulus, or impulse, or nerve fibers, but upon the distal nerve ending, whose function it is to excite nerve cells into specific activity. Theories as to the nature of the impulse, or nerve conduction, are enormous, but facts that would substantiate, are neither many nor lacking in contradiction. Doubt as to its being of a chemical nature is derived from the facts that under normal conditions nerve fibers are incapable of fatigue; that no heat is produced as a result of the passage of an impulse, and that there is no conclusive evidence of metabolism. On the other hand, experiments have shown that nerve fibers have become fatigued. Garten has shown that one nerve, the olfactory of the pike, when stimulated by induction shocks, with an interval between the stimuli of as much as .27 seconds, gives evidence of fatigue, since its action current as measured by the capillary electrometer, diminishes in extent quite rapidly and recovers after a short time. It has also been found that, while a nerve deprived of oxygen loses its irritability, this event occurs more promptly if the nerve is constantly stimulated. Tashiro reports that by means of a new method, which is capable of detecting as little as .000,0001 g. of CO_2, he has been able to show that the resting nerve produces CO_2 and that its production is increased two and one-half times when the nerve is stimulated. The assumption, therefore, is that a sensitive and unstable substance existing within the axis-cylinder is upset by the stimulus at the point stimulated, so that the energy thus liberated acts upon contiguous particles, and

so the disturbance is propagated along the nerve as a progressive chemical change which may be compared to the passage of a spark along a line of gunpowder. An opposite view is taken in the attempt to prove that the stimulus passing through the nerve fiber is electrical in nature. This would agree with the quickness of recovery from fatigue and the slight evidence of metabolic changes that many observers have recorded as the result of stimulation of nerve fibers. On the positive side it is found that if a cut nerve is connected with its sheath through a delicate galvanometer, an electric current flows from the cut end to the sheath. Furthermore, with a core model consisting of a glass tube with a core of platinum wire and a sheath of solution of NaCl .6 per cent, electrical phenomena can be obtained similar to those shown by the stimulated nerve. If an induction current, serving as a stimulus, is sent into one end of such an artificial nerve, and from the other end two leading off electrodes are connected with a galvanometer, then it can be demonstrated that an electrical charge is propagated along the model at each application of the stimulus. Moreover, such a moving electrical disturbance is the only objective phenomenon known to occur in the stimulated nerve, and it is assumed that it constitutes the nerve impulse. When this electrical disturbance reaches the end organ it initiates the chemical changes that characterize the activity of the organ. Corresponding to the central thread of the core conductor would be the neurofibrils within the axis-cylinder, and to the liquid sheath of less conductive material surrounding the central thread would be the perifibrillar substance. The fact that the rate of propagation of the nerve impulse is 123 meters per second, whereas that of an electric current is 186,000 miles per second, would lead us to believe that the current is not a direct but an intermittent one. Nernst supposes that the electrolytes contained in the axis-cylinder lie within membranous partitions which are impermeable to the passage of certain ions. When an electrical current is passed through a nerve it is conveyed by the dissociated electrolytes and, in consequence of the impermeable character of the septa, there will be a concentration of the positively charged ions on one side of the membrane and the negatively charged on the other. When the concentration of the ions reaches a certain point excitation occurs.

The visual and auditory sensations are now aroused. How are they to be transformed into memory-ideas? How and where are these to be retained? How are they to be reproduced? These are questions for an explanation of which we must turn to the psychologist.

Besides the sense and motor centers, which occupy comparatively small areas of the cortex, there are large areas occupied by cellular and fibrous network which form connections between the various sensory centers. These are called association areas. There are three groups; the frontal or anterior, the median or insular, and the posterior, extending to the occipital lobe and also laterally to the temporal lobes. Their general function is to unite the sense impressions into general knowledge. This function is indicated by the anatomic fact that they are connected with the various sense centers by tracts of association fibers, suggesting a mechanism by which the sense qualities from the separate sense centers may be combined to form a mental image of a complex nature. The posterior area is concerned especially in the organization of the

experiences founded upon visual and auditory sensations. The anterior area is especially concerned in the organization of experiences based upon internal sensations. The cortex of Reil is probably a part of the speech area, both on the motor and sensory sides. It is in these association centers that our memory records of past experiences and their connections are laid down in some material change in the network of the nerve cells and fibers. Experiments have shown that lesions in the sense centers or in the association areas connected with the sense centers produce defects of memory corresponding to the sensations which such sense centers normally produce. Auditory aphasia, or loss of power to understand spoken language, is due to injury to the auditory center, or to the immediately contiguous areas of the temporal lobes. Alexia, or loss of power to understand written or printed language, is due to a lesion involving the inferior parietal convolutions. Olfactory amnesia, or defects in the sense of smell, follow lesions in the distal portion of the hippocampal gyrus. Studies of mental pathology offer adequate proof that memory processes, retention, and reproduction, are dependent upon the sense centers and the association areas connected with them.

It is held by some that memory is a universal function of organized matter. Any change that may be suffered by any substance tends to persist. Every sight and every sound leave impressions on the brain. When subsequently the same or a similar sight or sound is perceived it touches the same spot, as it were, in the brain.

The reproduction or revival of previous impressions may take place by an act of will or by similar impressions or associations of the original impression. When a revival takes place by a repetition of the same sensory impulse we probably have a repetition of the same process in the same nerve elements. When one revives a memory in ideation only, there is also a repetition of the same nerve process in the same nerve elements that took place when the object was originally before the eyes. In addition, we have the laws of association; namely, that memories are recalled by the recurrence of sensations, or revival of memories associated with the original memories, through contiguity, succession, similarity, or contrast.

When we recognize an old memory, we locate it in time and we do this by mentally determining its relation to the present time; in fact, we could have no idea of time if it were not for memory; all would be present; we would have no past, nor could we anticipate the future.

Another conception of the nature of the change induced in nerve cells as a result of previous stimulation has been suggested by Matthews. According to this investigator, there are reasons for believing that when a nerve cell is touched by an impulse a change in its chemical composition or in the arrangement of its molecular structure is produced. An interesting and highly suggestive parallel is drawn by Matthews between the manifestations exhibited by linseed oil and brain cells. One of the chief constituents of the brain cell is cephalin, which contains unsaturated fatty acids of the linoleic acid type. Linseed oil likewise contains linoleic acid. When linseed oil is exposed to light or ultraviolet rays in the presence of air in a closed flask provided with a mercury manometer, for the first twenty-four to thirty-six hours nothing seems to happen. Then slowly

the oil begins to oxidize, and it oxidizes at a constantly accelerating pace so that the oxygen is used up in the flask and the negative pressure may be measured by the manometer. It is as though the oil had to be taught by the light to oxidize itself and learns to oxidize better and better. It may be shown that the oil possesses something like a memory. If after sixty hours' illumination, when the oil is oxidizing at a fairly rapid rate, the illumination is discontinued, it will be found that the oxidization no longer waits twenty-four hours before beginning, but the stimulation of the lamp is effective within an hour or more; the oil acts as though it remembered the teaching of a previous illumination, and now oxidizes at a more rapid rate. The processes which the oil undergoes are autocatalytic. They consist in the persistence in the oil of an intermediary catalytic oxidization product. If it were possible that an impulse coming into certain cells caused in those cells the formation of a persistent autocatalytic intermediary oxidization product, a physical basis of memory might be given. However, as it is, our knowledge of the underlying basis of memory is at best imperfect, and only through a continuous and untiring study can we hope to attain a complete solution of this interesting question.

—*V. C. V.*

United States Civil Service Examination for Bacteriologist, June 22, 1920.

The United States Civil Service Commission announces an open competitive examination for bacteriologist. A vacancy at St. Elizabeth's Hospital, Washington, D. C., at $2,500 a year, with temporary increase granted by Congress of $20 a month, and maintenance, and vacancies in positions requiring similar qualifications, at this or higher or lower salaries, will be filled from this examination, unless it is found in the interest of the service to fill any vacancy by reinstatement, transfer, or promotion.

All citizens of the United States who meet the requirements, both men and women, may enter this examination; appointing officers, however, have the legal right to specify the sex desired in requesting certification of eligibles. For the present vacancy male eligibles are desired.

Competitors will not be required to report for examination at any place but will be rated on the following subjects, which will have the relative weights indicated on a scale of 100: (1) Physical ability, 10. (2) Education, training and experience, 90.

UNDER THE SECOND SUBJECT COMPETITORS WILL BE RATED UPON THE SWORN STATEMENTS IN THEIR APPLICATIONS AND UPON CORROBORATIVE EVIDENCE.

Applicants must have graduated from a recognized medical college, and have had at least five years' bacteriologic and sanitary experience, including experience in the following lines: (a) Performance of autopsies; (b) Wassermann work; (c) Urinalysis; (d) Blood counts; (e) Sputum examination.

Special credit will be given for graduate courses in bacteriology, pathology and general sanitary work in the U. S. Army or in domestic or foreign universities; also for experience in similar work in a hospital for the insane.

Applicants must have reached their twenty-sixth birthday on the date of the examination. Age limits do not apply to persons entitled to preference because of military or naval service.

Applicants must submit with their applications their unmounted photographs, taken within two years, with their names written thereon. Proofs or group photographs will not be accepted. Photographs will not be returned to applicants.

Applicants should at once apply for Form 1312, stating the title of the examination desired, to the Secretary of the Fourth Civil Service District, 8th and E Streets, N. W., Washington, D. C., or to the Secretary of the United States Civil Service Board, Customhouse, Boston, Mass., New York, N. Y., New Orleans, La., Post Office, Philadelphia, Pa., Atlanta, Ga., Cincinnati, Ohio, Chicago, Ill., St. Paul, Minn., Seattle, Wash., San Francisco, Calif., or Old Customhouse, St. Louis, Mo.

Applications should be properly executed, excluding the county officer's certificate, but including the medical certificate and must be filed with the Secretary of the Fourth Civil Service District, 8th and E Streets, N. W., Washington, D. C., prior to the hour of closing business on June 22, 1920.